As agreed upon by the Trustees and BP, all samples collected for contaminant analysis during the sampling plan described below will be sent to Alpha Analytical Laboratory, unless they are designated to be archived. Samples for other analyses, if not archived, will be sent to the laboratories indicated in the plan below.

Each laboratory shall simultaneously deliver raw data, including all necessary metadata, generated as part of this work plan as a Laboratory Analytical Data Package (LADP) to the trustee Data Management Team (DMT), the Louisiana Oil Spill Coordinator's Office (LOSCO) on behalf of the State of Louisiana and to BP (or ENTRIX behalf of BP). The electronic data deliverable (EDD) spreadsheet with pre-validated analytical results, which is a component of the complete LADP, will also be delivered to the secure FTP drop box maintained by the trustees' Data Management Team (DMT). Any preliminary data distributed to the DMT shall also be distributed to LOSCO and to BP (or ENTRIX on behalf of BP). Thereafter, the DMT will validate and perform quality assurance/quality control (QA/QC) procedures on the LADP consistent with the authorized Quality Assurance Project Plan, after which time the validated/QA/QC-ed data shall be made available simultaneously to all trustees and BP (or ENTRIX on behalf of BP). Any questions raised on the validated/QA/QC results shall be handled per the procedures in the Quality Assurance Project Plan and the issue and results shall be distributed to all parties. In the interest of maintaining one consistent data set for use by all parties, only the validated/QA/QC-ed data set released by the DMT shall be considered the consensus data set. In order to assure reliability of the consensus data and full review by the parties, no party shall publish consensus data until 7 days after such data has been made available to the parties. Also, the LADP shall not be released by the DMT, LOSCO, BP or ENTRIX prior to validation/QA/QC absent a showing of critical operational need. Should any party show a critical operational need for data prior to validation/QA/QC, any released data will be clearly marked "preliminary/un-validated" and will be made available equally to all trustees and to BP (or ENTRIX on behalf of BP).

All materials associated with the collection or analysis of samples under these protocols or pursuant to any approved work plan, except those consumed as a consequence of the applicable sampling or analytical process, must be retained unless and until approval is given for their disposal in accordance with the retention requirements set forth in paragraph 14 of Pretrial Order # 1 (issued August 10, 2010) and any other applicable Court Orders governing tangible items that are or may be issued in MDL No. 2179 IN RE: Oil Spill by the Oil Rig "DEEPWATER HORIZON" (E.D. LA 2010). Such approval to dispose must be given in writing and by a person authorized to direct such action on behalf of the state or federal agency whose employees or contractors are in possession or control of such materials.

This plan will be implemented consistent with existing trustee regulations and policies. All applicable state and federal permits must be obtained prior to conducting work.

Approval of this work plan is for the purposes of obtaining data for the Natural Resource Damage Assessment (NRDA). Parties each reserve its right to produce its own independent interpretation and analysis of any data collected pursuant to this work plan.

APPROVED:

FOR KOLAND GUIDRY

Louisiana Trustee Representative: Date 2/22/11

BP Representative: Date Feb 11, 2011

NOAA Trustee Representative Date 3/9/11

(on behalf of all other trustees)

Mississippi Canyon 252 Spill
Oyster Sampling Transition Plan Summary
October 2010 to April 2011

February 3, 2011

Objective/Purpose: After reviewing the progress of the field efforts implemented under the Phase I work plan and its amendments, the oyster technical working group (Oyster TWG) has determined that there is a clear need to collect further data to document the potential exposure of oysters to oil and dispersants released into the environment as a result of the Deepwater Horizon Oil Spill, as well as documenting potential injury to oyster resources as a result of such exposure.

The Phase I sampling plan focused on oyster collection at historic collection locations of the States' resource management agencies (~36 sites in LA, 15 in MS, 12 in AL, and 12 in FL). This sampling was not intended to necessarily provide a sample size adequate for characterizing the extent of contaminant exposures and potential incident or response-related injuries across the resource population. In addition, the persistence of spill-related contaminants in the environment continues to pose a threat of potential ongoing exposure of oysters to these contaminants and potential injury resulting from such exposure. The Oyster TWG plans to sample additional locations in a more rapid manner, which will result in additional quantitative contaminant samples of oysters and sediments, larval densities, and recruitment of spat as well as qualitative abundance estimates of adult oysters (catch per unit effort (CPUE) data from oyster dredges). The transition plan will therefore allow for collection of ephemeral data that may be of direct use to establish exposure criteria for oysters and oyster condition. This additional sampling is a time-sensitive element of the pre-assessment because exposure metrics (oyster contaminant burden, sediment contamination) will likely change between late October and next year. Because of these potential changes it would be useful to obtain data on oyster populations and recruitment levels beginning in the month of October.

An additional benefit of the transition plan is that it will provide a temporal bridge between completion of the Phase I (amended) sampling program and any injury assessment work the team may pursue in 2011. Specifically, this transition plan will allow for continued progress on identifying future sample locations within the area of known oyster habitat, including both mapped oyster reef and unmapped areas identified by State biologists to have a high probability of productive oyster habitat based on historic knowledge of industry activities and field observations of commercial harvest. Below is a summary of the key aspects of the transition plan:

- The plan focuses on known oyster habitat in Louisiana and Mississippi. Known oyster habitat includes both previously mapped oyster reef and unmapped areas identified by State biologists to have a high probability of productive oyster habitat.
- Sixty 600 meter x 600 meter grid cells (sites) have been chosen from this stratum in Louisiana and 10 sites have been chosen in Mississippi (see site selection procedures below).
- A set of three dredge replicates per site are planned to document presence/absence of live oyster[1] as well as provide quantitative enumeration of oyster abundance.
- Live oysters, if present, will also be collected from these dredge samples and analyzed for contaminant burden and gonad/disease condition metrics.
- Sediment samples for contaminant analysis will also be collected at each site if appropriate substrate is present. Two composite samples of two samples each may be prepared in the lab from four individual samples collected per cell independently in the field.
- Sites found to have live oyster resource and/or recently dead[2] resource may be sampled in a more quantifiable manner with quadrats at a later date.
- Oyster recruitment and larval supply metrics may be measured during up to three sampling rounds at these sites.
- Data produced by the Louisiana Department of Wildlife and Fisheries (LDWF) stock assessment sample collection efforts undertaken at Tier 1 sites in June and July of 2010 may also be used for comparison against the dredging undertaken for the purposes of this transition work plan.

Estimated samples from this activity (see Tables 1 and 2):

- 70 dredge surveys (one set of three replicates per site);
- 70 sets of sediment samples (two potential composites per site; 140 samples total);
- 70 composite oyster tissue samples (one per site, up to 6 market-sized oysters analyzed (or equivalent) per sample);
- 70 oyster gonad/disease/condition samples (one per site, up to 10 market-sized oysters analyzed per sample);
- 70 sets of larval samples (three sampling events by year end 2010; 210 samples total); and

[1] Oysters from each replicate will be enumerated into three size categories: spat (less than 1 inch shell height); seed or juvenile oysters (between 1 and 3 inches); and market size or "legal" oysters (greater than 3 inches).

[2] Recently dead oysters are defined as those with articulated shells with no evidence of fouling inside the shell.

- 70 sets of recruitment samples (two sampling events by year end 2010; 140 samples total).

Site Selection

A sample of 60 grid cells (sites) in Louisiana of size 600 x 600 meters has been probabilistically selected using the generalized random tessellation stratified (GRTS) sampling procedure (Figures 1-5).[3] Additionally, a sample of 10 grid cells (sites) in Mississippi of the same size has been selected (Figure 6).[4] If a full assessment plan is implemented in 2011, these 70 grid cells (sites) may continue to be sampled. The probabilistic sample of sites from the GRTS sampling procedure should be representative of the range of conditions within the sample frame with respect to degrees of oiling (including non-oiled locations), areas affected by freshwater diversions instituted by Louisiana as part of response actions, and areas affected by neither oiling nor freshwater diversions.

Available data regarding oil exposure or response actions will be used to guide any additional sample site selection as such data become available. Such data may include, but are not limited to, an index of surface oil exposure as generated from satellite imagery for the period of April 22 through August 1, 2010, the distance to nearest oiled shoreline (by category) as generated from the Shoreline Contamination Assessment Team (SCAT) data, a measure of oil exposure generated from tissue contaminant data collected for food safety investigations since the start of the incident, observations of submerged oil through either response or NRDA actions, and identification of freshwater diversions. With the sample sizes proposed, the sample site distribution with respect to any given range of oil exposure or response action may significantly deviate from the sample frame distribution. A selection of sample sites that deviates significantly from the known range of exposure or response actions would impede modeling efforts due to the lack of samples along some part of the distribution (range). An example distribution for continuous data is presented in Figure 7. In this case, additional site selection in the range of exposures not represented (strata) may be warranted to ensure enough points and range exist in the predictor variables for any potential modeling efforts during data analysis. For categorical data (e.g. freshwater diversions), a minimum of 10 sites within each category is desirable. If warranted, additional sites will be selected by referring to the GRTS list of potential sites and selecting in order the next sites on the list with the oil exposure or response action

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[3] McDonald,T. 2004. GRTS for the Average Joe: A GRTS Sampler for Windows. http://www.west-inc.com.

Stevens, D. L. and Olsen, A. R. 1999. Spatially restricted surveys over time for aquatic resources. *Journal of Agricultural, Biological, and Environmental Statistics*. 4:415-428.

Stevens, D. L. and A. R. Olsen. 2004. Spatially balanced sampling of natural resources. *Journal of the American Statistical Association*. 99:262-278.

[4]Mississippi noted that sites selected in Phase I tended to fall on the outer edges of the oyster reef.

values needed to supplement the initial sample site selection. Sample sites selected as part of this additional site selection procedure provide a valid probabilistic sample from the respective strata, may be used in generating any models, and may be used in making statistical inferences to the strata. The transition plan consists of four types of sampling events:

1. **Recruitment Sampling** – This includes collecting larval samples, and deploying and collecting settlement plates for recruitment analysis (see Appendix A, Sections B and C for detailed recruitment sampling standard operating procedures (SOP)). Up to three sampling events are planned between October and December 2010 at approximate three week intervals, as weather and schedule allows.

2. **Mapping** –Cells may be mapped using tickle chains, cane poles, or other appropriate methods in combination with GIS software (see Appendix A, Section A for detailed mapping SOPs).

3. **Dredging** – Using the random contact points generated following the mapping effort in areas determined to have appropriate substrate, a set of three dredge replicates may be collected to enumerate live and recently dead oyster (as CPUE) and to collect an oyster sample at each site if appropriate habitat and oysters are present (see Appendix A, Sections D, F and G for detailed dredging SOPs and SOPs for contaminant, gonadal and disease analyses). Field teams will bag a sufficient number of dredges to retain the first 20 live, market-size oysters they encounter for sample preparation by the intake laboratory. If the initial three dredges do not collect 20 suitable market-sized oysters (or equivalent, as described in the dredge SOP), dredging may continue for up to a total sampling time of 2 hours at the additional contact points provided. In addition to enumerating live and recently dead oysters and preparing contaminant and disease/gonad samples, the intake lab team will record qualitative descriptions of any dead oyster and shell collected in the dredges, as well as any observations concerning oiling and overall reef condition.

4. **Sediment Sampling** – Sediment samples will be collected at contact points randomly selected for each site if appropriate substrate is present. Four spatially independent samples will be collected per cell and potentially combined into two composite samples. See Appendix A, Section E for the detailed sediment sampling SOP, which is based on the findings of the attached draft letter report, "Draft Sediment Sampling Method Evaluation, NOAA NRDA Oyster Technical Work Group, Mississippi Canyon 252 Spill," dated January 12, 2011 and prepared by Stephen Emsbo-Mattingly of Newfields.

All samples from the sampling efforts will be processed at Dauphin Island Sea Lab, with the exception of samples for gonad/disease analysis, which will be sent to the University of New Orleans for analysis. Larval samples and settlement plates will be processed at Dauphin Island Sea Lab and archived for potential future analysis. Oysters for contaminant analysis will also be

archived at Dauphin Island Sea Lab. Sediment samples will be shipped to Alpha for analysis. Both collection and lab processing will follow the detailed SOPs included in Appendix A of this document.

Health and Safety

- **The team leader and field crew parties should have completed all applicable health and safety training as directed by NOAA or state agency oil spill policy.**

- **All field team members must complete the NOAA safety training and documentation requirements** as set forth in "Safety Requirements for All Personnel Working on NOAA-led NRDA teams for MS Canyon 252 Incident".

- **All field team members should read all of the safety documents posted on the case site.** [5]
  Exception: if site collection activities do not include use of a boat or helicopter, then familiarity with the safety documents for these vehicles is not required.

- **Each field team must submit a plan, not later than the night prior to going into the field.** This plan must specify:
  - The team leader;
  - Names of all team members;
  - The sampling location(s)-- please use the grid coordinates as provided to your team by NOAA NRDA Field Ops staff or the NRDA Oyster Sample Location Coordinator;
  - What kind of sampling they are doing;
  - Expected arrival time at sampling area (daily);
  - Expected departure from sampling area (daily);
  - Team deployment date;
  - Team return date.

  This information may be reported in one of two ways:
  1. Fill out the Excel spreadsheet "Team Member Information Form – Sampling and Safety.xls"[6] and send it to [redacted] Please use one tab for each team.
  2. If you cannot submit this spreadsheet electronically, you can call in and report the information using this number: [redacted]

[5] Previously available on the case ftp site: [redacted]
Future field efforts should consult the Safety Protocols directory within the Documentation area of [redacted]

[6] Previously available on the case ftp site: [redacted]
Future field efforts should consult the Safety Protocols directory within the Documentation area of [redacted]

- **Field teams must adhere to all procedures set forth in the most recent version of the MC252 Site Safety Plan in effect at the time of their field work (currently the 1/28/2011 version).**[7]

- **If participating in a cruise:** Each cruise may have additional required health and safety procedures, which must be observed.

- **Diving:** SCUBA or surface-assisted diving, where used for sampling, will be conducted in accordance with existing Trustee dive safety programs.

Cost Estimate

The cost associated with this level of field effort is $566,717. The Oyster TWG also intends to analyze sediment samples collected during this study for contaminant concentrations, which would be associated with an additional $210,000 in costs. Any oysters collected for tissue contaminant analysis and water samples for quantitative polymerase chain reaction (PCR) of larvae collected under this field effort may be archived for potential analysis at a later date. Gonad/disease samples will not be archived due to time sensitivity in completing these analyses. An additional cost of up to $131,950 could be incurred assuming all samples are eventually analyzed. The total cost of the study if all samples were analyzed would then be $908,667. For additional detail concerning the cost estimate, please consult Table 3 and the attached Excel file, "Transition Plan Cost Matrix_12.17.10.xlsx".

The Parties acknowledge that this budget is an estimate, and that actual costs may prove to be higher due to a number of potential factors. BP's commitment to fund the costs of this work includes any additional reasonable costs within the scope of this work plan that may arise because of any contingencies. The trustees will make a good faith effort to notify BP in advance of any such contingencies.

[7] Previously available on the case ftp site: [redacted]
Future field efforts should consult the Safety Protocols directory within the Documentation area of [redacted]

Table 1. Proposed Phase I metrics.

Metric	Proposed Frequency of Sampling
Effect Metrics	
Oyster abundance (qualitative)	One event
Disease	One event
Gonadal condition	One event
Larval abundance (#/L)	Up to three events (spaced three weeks apart)
Larval settlement	Up to two events (spaced three weeks apart)
Exposure metrics	
Tissue concentrations	One event
Sediment concentrations	One event
Oiling observations (qualitative)	Collected on each site visit

Table 2: Estimated Sampling Activity for Transition Plan

Metric	Method	N = Sample Sizes (Potential # of sites)				*Estimated subsamples* per site	Estimated subsamples per event	Freq. of sampling	Estimated Total # of subsamples
		LA	*MS*	*FL*	*AL*				
Site Mapping and Initial Visit	Poling	60	10	0	0	1	NA	1	NA
Oyster Larvae	Water sample	60	10	0	0	5	350	3	1,050
Oyster Settlement	Settlement plate	60	10	0	0	3 plates	210	2	420
Oyster Gonadal, Condition and Disease	Oysters	60	10	0	0	10 oysters	700	1	700 oysters
Tissue contaminant analysis	Oysters	60	10	0	0	6 oysters (1 composite)	420 – 700 oysters (70 composites)	1	420 - 700 oysters (70 composites)
Sediment Contaminant analysis	Sediment	60	10	0	0	2 composites per grid cell	140	1	140

Table 3. Costs for Oyster Sampling Transition Plan.

Item	Unit cost	Units	Units	Costs (per event)	# (of events)	Total cost
FIELD SAMPLING/PROCESSING						
Larval/Settlement Plate Sampling				59,500	3	178,500
Personnel	[redacted]	Person days	[redacted]	33,600		100,800
Boat charges	1,600	Days	14	22,400		67,200
Supplies	250	Days	14	3,500		10,500
Mapping				148,750	1	148,750
Personnel	[redacted]	Person days	[redacted]	84,000		84,000
Boat charges	1,600	Days	35	56,000		56,000
Supplies	250	Days	35	8,750		8,750
Dredging				59,500	1	59,500
Personnel	[redacted]	Person days	[redacted]	33,600		33,600
Boat charges	1,600	Days	14	22,400		22,400
Supplies	250	Days	14	3,500		3,500
Sediment Sampling				99,167	1	99,167
Personnel	[redacted]	Person days	[redacted]	56,000		56,000
Boat charges	1,600	Days	23	37,333		37,333
Supplies	250	Days	23	5,833		5,833
Larval/Settlement Plate Processing				11,900	3	35,700
Personel	[redacted]	Person days	[redacted]	8,400		25,200
Supplies	5	Samples	350	1,750		5,250
Shipping and archive charges	5	Samples	350	1,750		5,250
Sediment Processing				1,400	1	1,400
Supplies	5	Samples	140	700		700
Shipping and archive charges	5	Samples	140	700		700
Dredge Processing				35,700	1	35,700
Personel	[redacted]	Person days	[redacted]	33,600		33,600
Supplies	5	Samples	210	1,050		1,050
Shipping and archive charges	5	Samples	210	1,050		1,050
Cooler Rental	8,000					8000
Field Sampling/Processing Total						**$566,717**
LABORATORY ANALYSIS						
Sediment Contaminants	1,500	sample	140	210,000	1	210,000
Oyster Contaminant	1,500	sample	70	105,000	1	105,000
Disease and Gonad	10	sample	70	700	1	700
DNA PCR	25	sample	350	8,750	3	26,250
Laboratory Total						**$341,950**
TOTAL						**$908,667**

Figure 1. Transition Plan Sampling Locations in Louisiana (CSA 1-2)

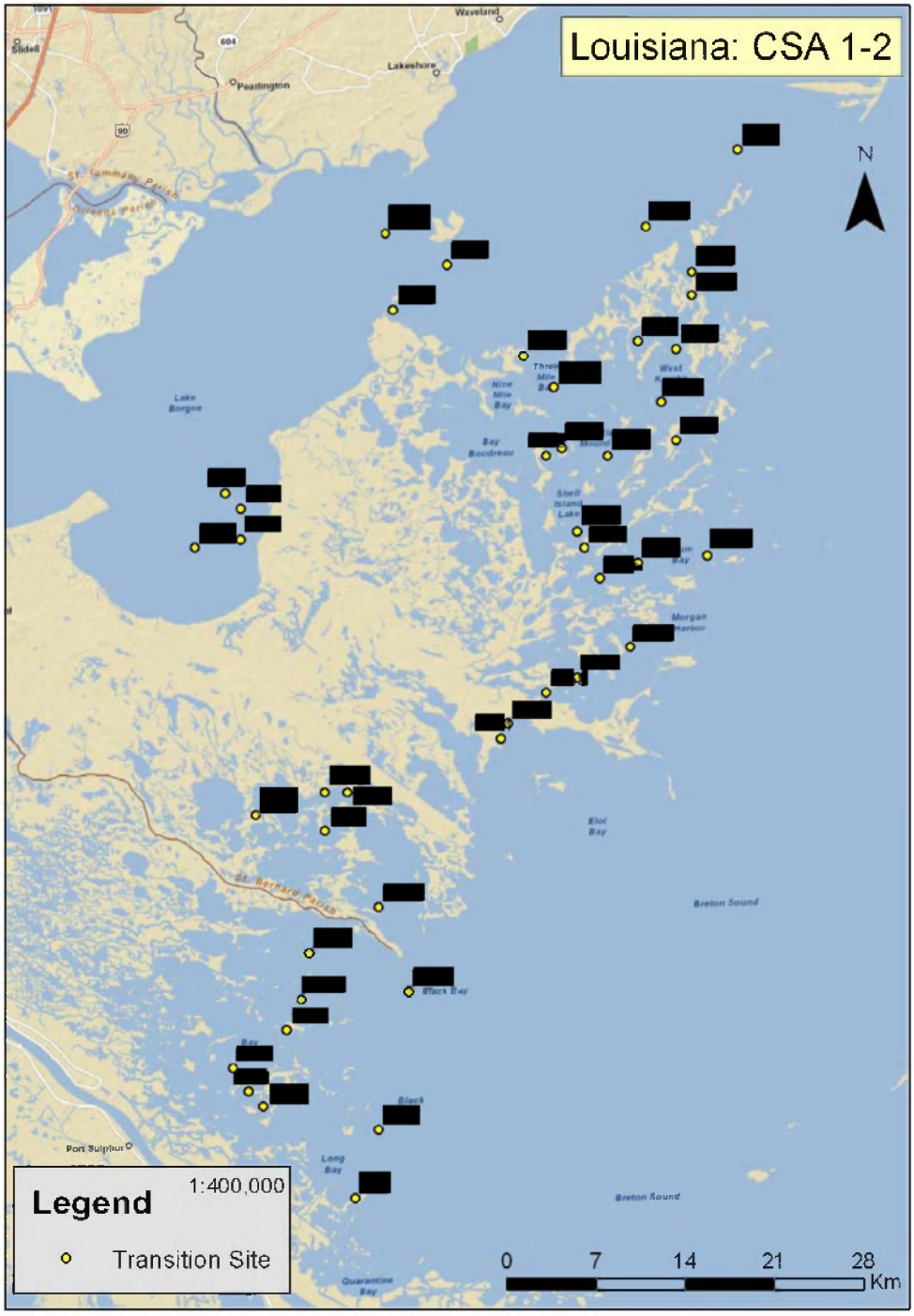


Figure 2. Transition Plan Sampling Locations in Louisiana (CSA 3)

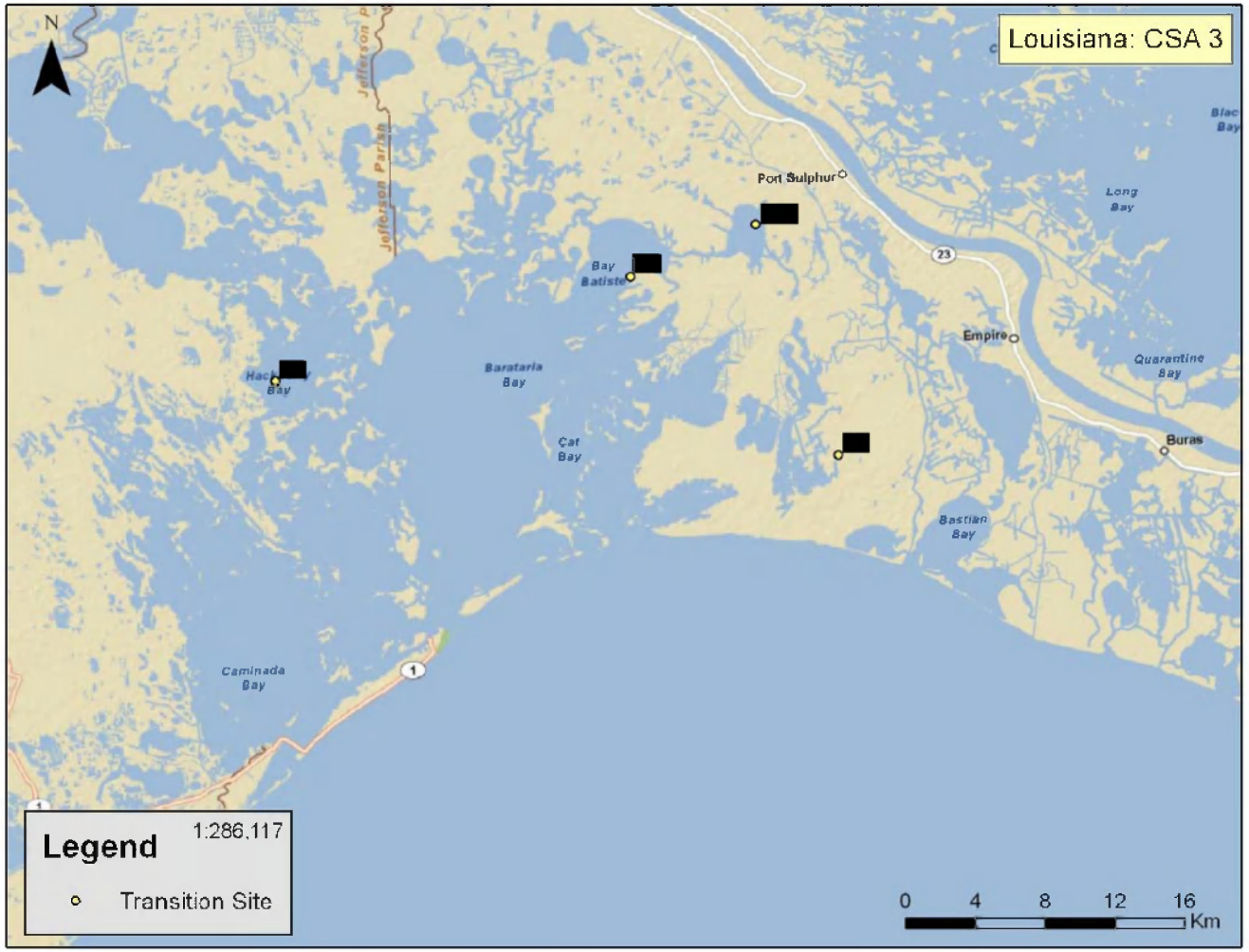


Figure 3. Transition Plan Sampling Locations in Louisiana (CSA 4-5)

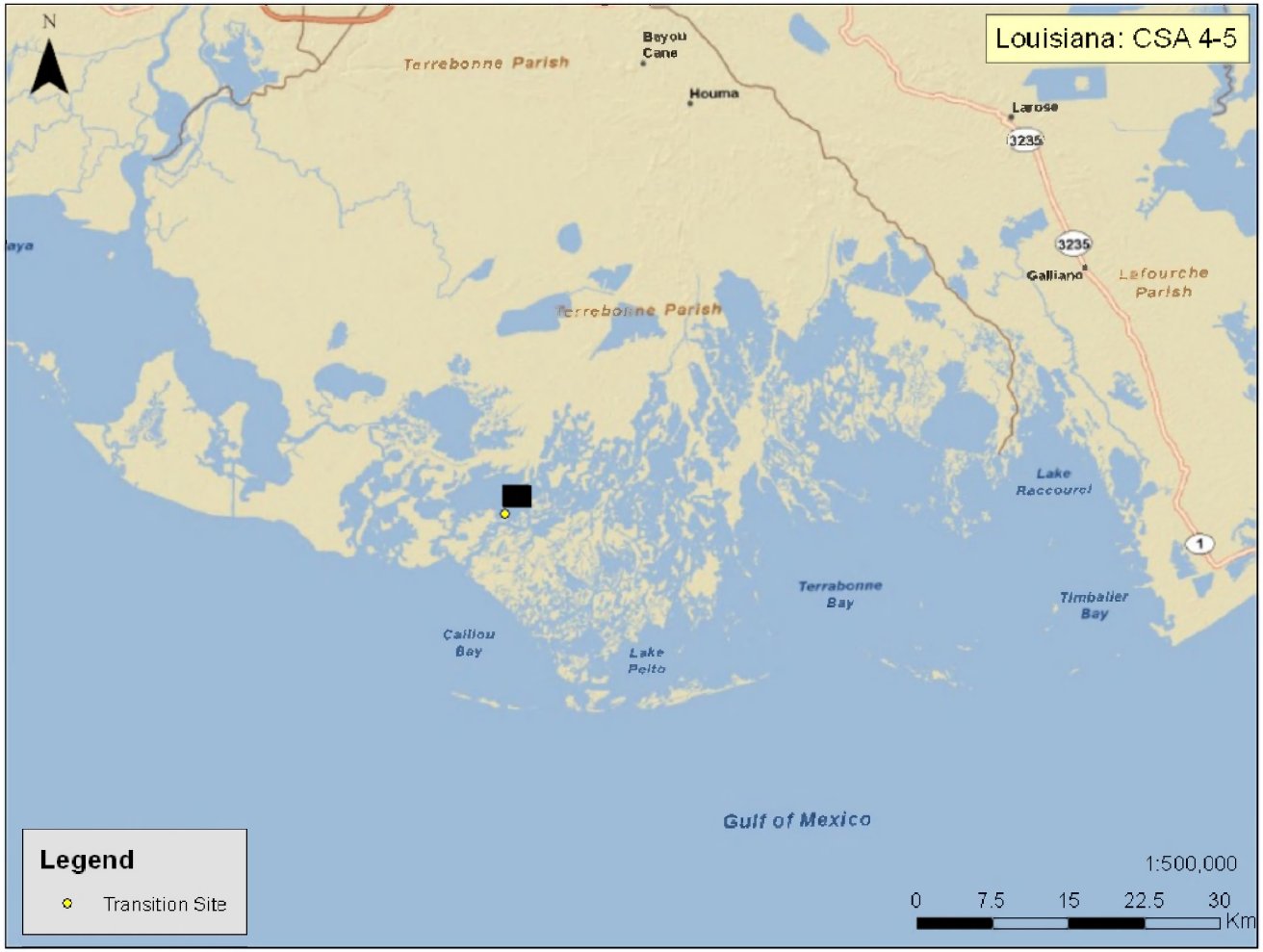


Figure 4. Transition Plan Sampling Locations in Louisiana (CSA 6)

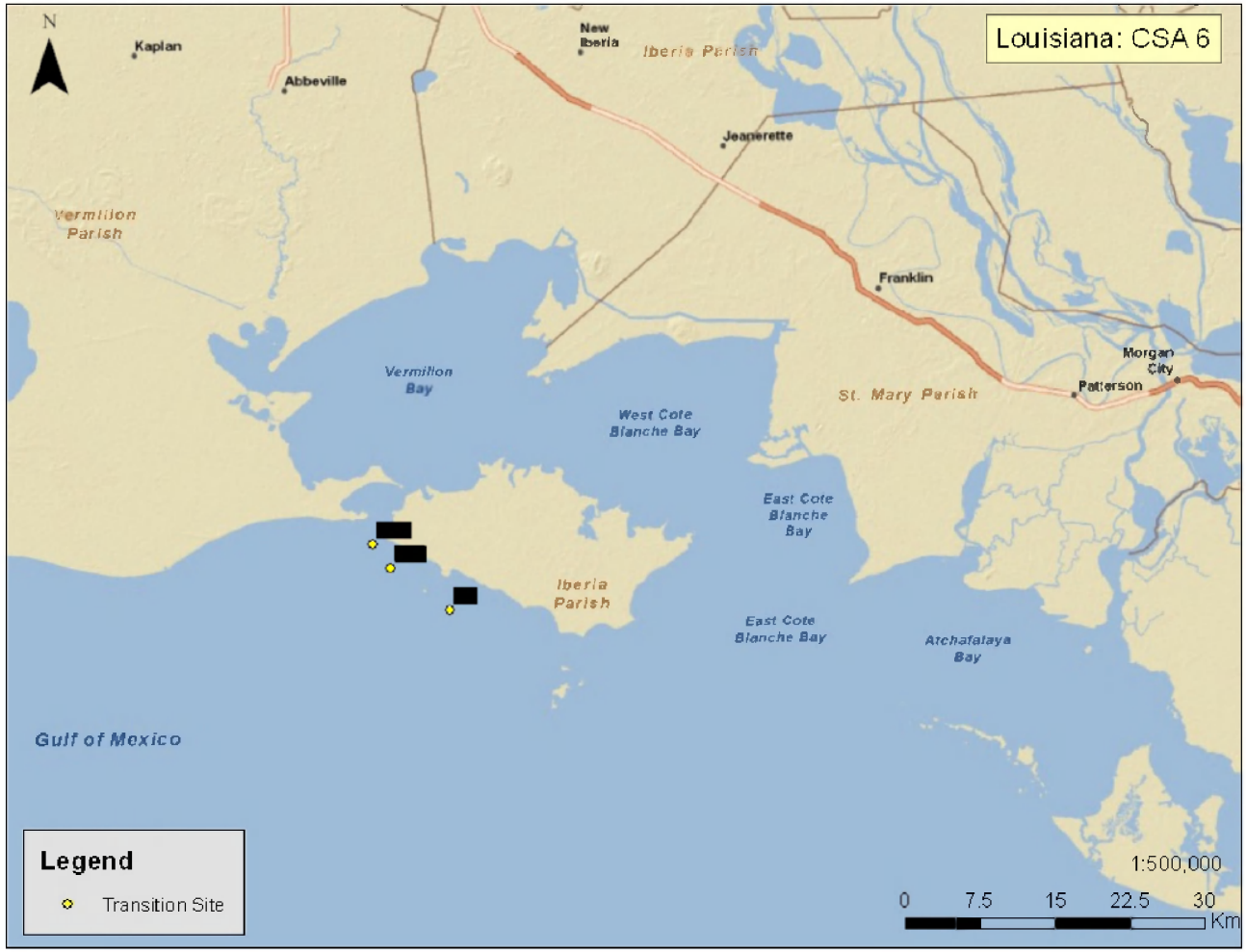


Figure 5. Transition Plan Sampling Locations in Louisiana (CSA 7)

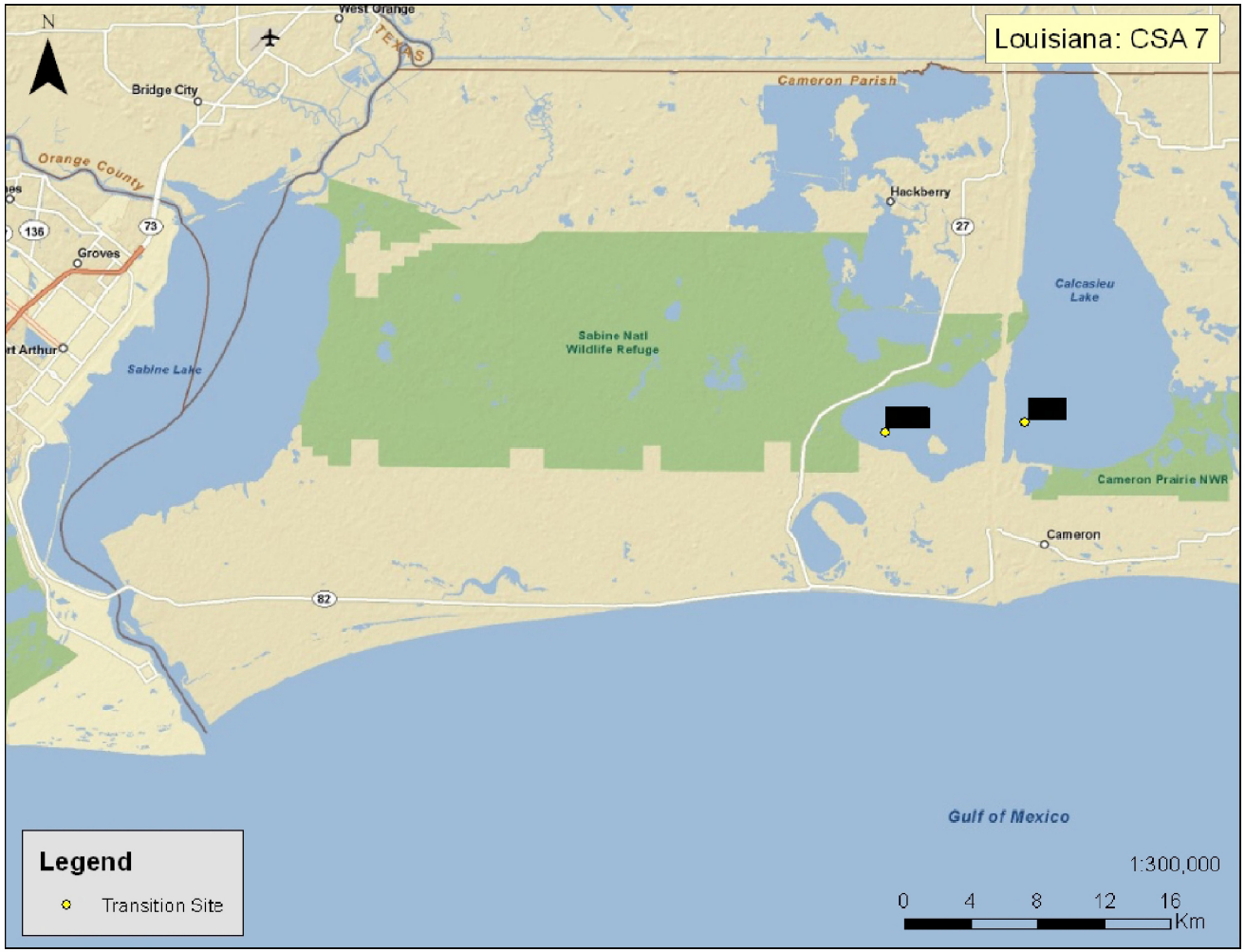


Figure 6. Transition Plan Sampling Locations in Mississippi

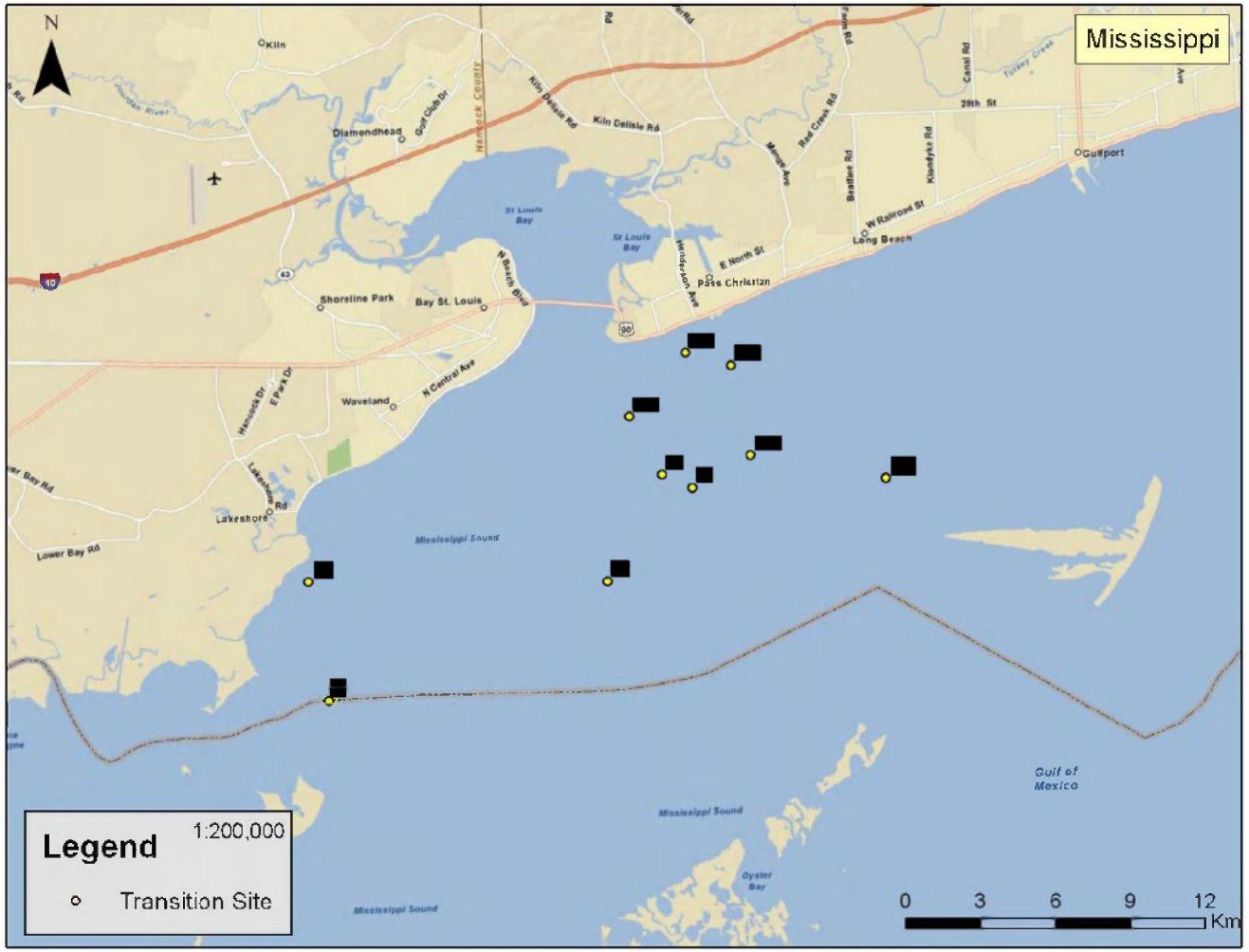
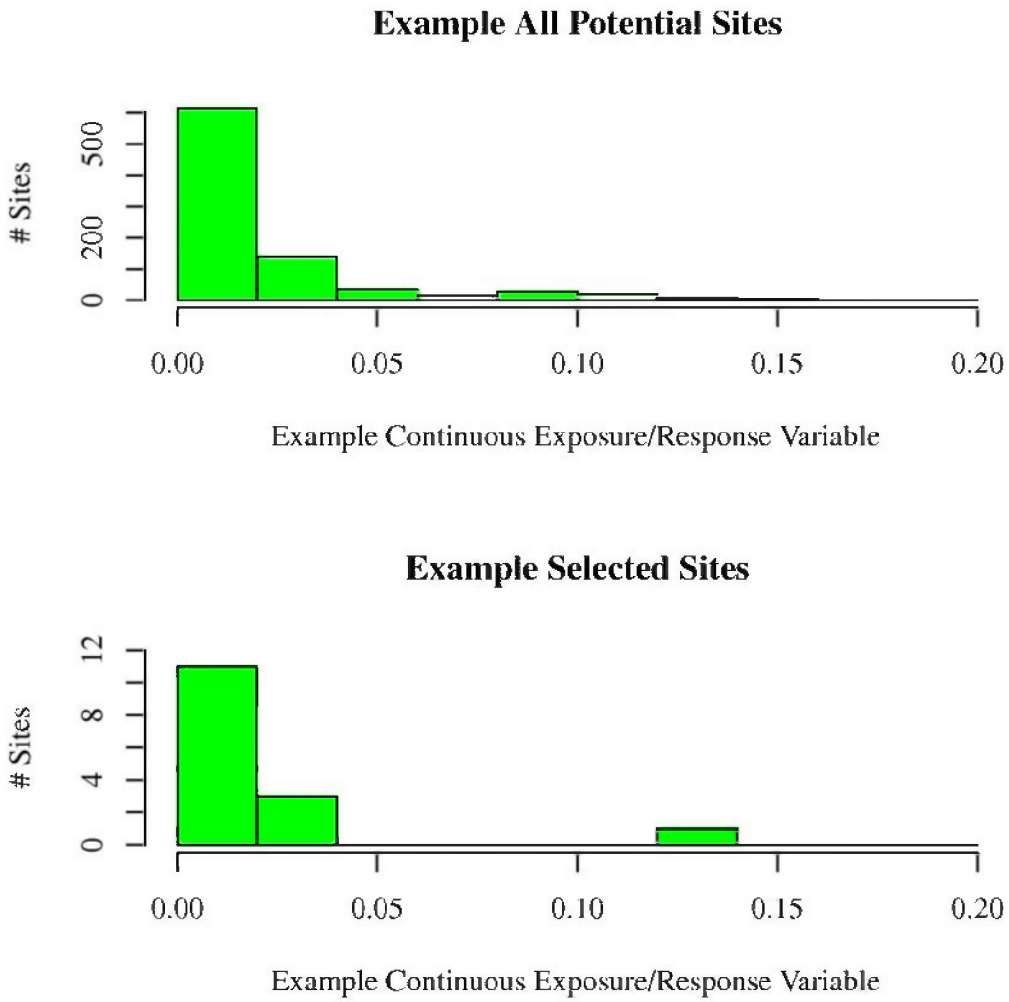


Figure 7. Oiling Exposure Example Distribution



APPENDICES

Appendix A: Detailed Standard Operating Procedures (SOPs)

Appendix B: Oyster Sample ID Naming Convention

Appendix C: Oyster Transition Plan Field Forms

Appendix A: Detailed Standard Operating Procedures (SOPs)

A. Site mapping - Initial Site Surveys

Each sampling location may be mapped in the field with the aid of a GPS device. Up to eight transects with an approximate north/south orientation running the length of the grid cell (uniformly spaced with a random starting point for the first transect and pre-programmed into the GPS device) may be surveyed. Teams can visually inspect if conditions allow; alternatively, they may either pull a chain (e.g., 1/4 inch galvanized links) or periodically prod with a long pole (approximately 1/2 inch diameter) along the transect, allowing the field scientist to feel or hear the 'tickle' of the chain on oyster habitat or feel the bottom substrate with the pole. If using the pole, teams should prod the bottom surface at regular intervals approximately once every 10 seconds along the length of the transect, should conditions allow. Teams should use pole of sufficient length to reach the maximum depth expected in the waters being mapped (e.g., an 18 foot cane pole in MS waters). Poling will be conducted using bamboo, PVC or metal poles. The boat should continuously move along the transect at a slow speed, just above idle (e.g., 2 knots); teams should prod with the pole approximately every 10 seconds to determine bottom type. The bottom surface type will be called each time it changes and the field teams will record this information, along with the coordinates on the mapping field form. Teams will record the coordinates and type of bottom surface encountered starting at the beginning of the transect, and each time the bottom surface changes. Teams should record waypoints on the GPS device, and record coordinates and bottom surface type on the mapping field form. At the end of each transect, teams should record final coordinates on the field form and create a waypoint on the GPS device. If a transect is intersected by a land mass or other obstruction, teams should record stopping and restarting points on the field form and GPS device. Bottom surface type is to be classified as follows:

Bottom Surface Type	***Categories***	***Brief Description***
Type I	Soft Mud	Soft, slushy mud – would not support small pieces of cultch material
Type II	Moderately Firm Mud	Bottom that would support small pieces of cultch material
	Firm Mud or Sand	Compact muddy or sandy substrate
	Buried Shells	Shells buried under sediment
Type III	Exposed Shell	Single or scattered shells, or hard substrates such as clam shells, limestone, concrete aggregate, etc.
	Reef	Thick shell

Source: State of Louisiana Sampling Protocol for Projects in Public Oyster Areas, available at: http://dnr.louisiana.gov/crm/coastmgt/permitsmitigation/oyster/sampling-protocol.pdf

Field staff are responsible for transferring the mapping data from the field forms and GPS device to an Excel spreadsheet at the end of each field day. Once data have been entered into Excel, the field team leader is to deliver the Excel spreadsheet, a digital copy of the mapping field forms and the day's .gpx data file to the NRDA Oyster Sample Location coordinator, and CC the NRDA Field Operations email account (dwhnrdafieldops@gmail.com). Subsample points for dredging will be randomly located along the line segments of transects that intercept with Type III bottom surface type. Subsample points for sediment sampling will be randomly located along the line segments of transects that intercept with Type I bottom surface type or in Type II if Type I is not found within a site (see Figure A-1).

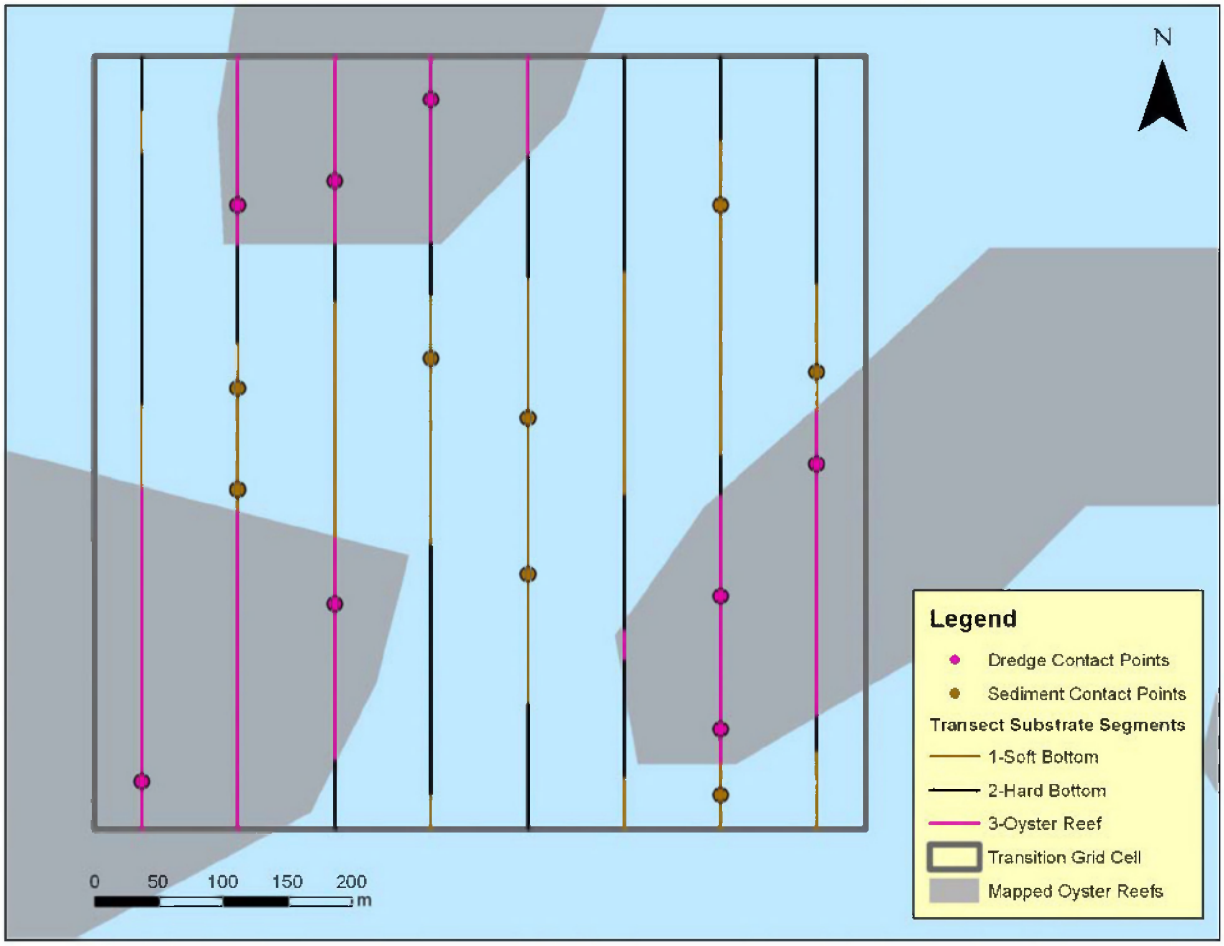


Figure A-1. Hypothetical grid cell with three reefs intersected by 8 transect lines.

B. SOP for Larval Concentrations

1. Objectives:

(a) Determine presence/absence of oyster larvae in the water column at each site as estimated by real-time quantitative polymerase chain reaction (QPCR) with larval and adult oyster DNA as standards.

(b) Quantify the abundance of total bivalve veliger larvae (non-oyster specific) (Fig. 2) in the water column.

Rationale: The eggs and larvae of many bivalves are indistinguishable using microscopy, but direct counting provides an absolute metric for the number of bivalve larvae in the water column at the time of sampling. There is evidence that sampling through the water column may collect varied life stages: eggs, developing larvae, and presettlement pediveligers (those larvae with a developed foot). In and of itself, this provides a relative index for spatial variability in the plankton, and also a good estimate of oyster larval abundance because oysters can be expected to be a dominant component of the total bivalve larval population. Only pediveligers can reliably be identified visually. QPCR quantifies oyster larvae by measuring the number of gene copies for a specific gene in the sample, and relating that measurement back to a standard curve prepared with known concentrations of larvae.

PCR and QPCR are made possible by harnessing the activity of DNA polymerase (commonly referred to as '*taq*', after the organism from which it was isolated for this purpose). The reaction occurs at temperatures - ~94, ~60, and ~72 C. The process by which a machine is used to heat and cool the reaction to those temperatures permissive for DNA melting and DNA duplication is known as thermocycling.

QPCR is accepted universally as a means of quantifying microscopic organisms, and finds application in pathogen detection, human immunodeficiency virus HIV diagnosis, and estimating larval quantity in other marine mollusks.

See for example;

De Faveri, J., Smolowitz, R.M., and Roberts, S.B. 2009. Development and validation of a real-time quantitative PCR assay for the detection and quantification of *Perkinsusmarinus* in the eastern oyster, *Crassotreavirginica*. Journal of Shellfish Research **28**: 459-464.

Matejusova, I., McKay, P., McBeath, A.J.A., Collet, B., and Snow, M. 2008. Development of a sensitive and controlled real-time RT-PCR assay for viral haemorrhagicsepticaemia virus (VHSV) in marine salmonid aquaculture. Diseases of Aquatic Organisms **80**: 137-144.

Vadopalas.Brent, Bouma, J.V., Jackels, C.R., and Friendman, C.S. 2006.Application of real-time PCR for simultaneous identification and quantification of larval abalone. Journal of Experimental Marine Biology and Ecology **334**: 219-228.

Wight, N.A., Suzuki, J., Vadopalas, B., and Friedman, C.S. 2009.Development and optimization of quantitative PCR assays to aid *Ostrealurida* Carpenter 1984 restoration efforts. Journal of Shellfish Research **28**: 33-41.

2. Field Procedure

a. Materials
- Nisken or LaMotte Water Sampler (fixed volume, remote trigger and 1 L capacity)
- 1-L amber sample bottles
- Waterproof labels
- Bubble wrap bags
- Clear tape
- Sharpie
- Water quality meter and calibration solution
- Distilled water

b. Methods
- Label bottles in advance of sampling with NRDA sample code.
- Lower sampler into water column at the center of the cell and allow it to remain open for 1 minute; depending on the conditions, the Nisken may need to be secured to a pole to ensure that it reaches the bottom.
- Trigger to close. Record sampling time on field form.
- Collect 5 water samples:
    - Two approximately 2 inches beneath water surface
    - Two just above the bottom (touch bottom and raise 3-4 inches)
    - One mid-water (approximately half-way between top and bottom)
    - If the water depth is very shallow (< 4ft), collect three bottom samples and two surface samples.
    - Samples containing more than approximately two tablespoons of sediment should be discarded, and the sample recollected following a distilled water rinse of the sample jar to remove sediment.

- Close lids tightly, record sample time on label, and secure label with clear tape. Note bottom, mid-water or surface in field notes and label/sample name (-LB, - LM, -LS)
- Place jar in bubble wrap bag and store samples on ice.

3. Sample Intake Procedure

a. Materials
   - 95% Ethanol solution (EtOH). All chemicals must be reagent grade, and water should be filtered and deionized.
   - 35-micron sieve (prepare extras in case of rips)
   - 250-ml or 500-ml squirt bottles
   - 50-mL plastic centrifuge tubes

b. Methods

   1. Label one of the top and one of the bottom samples as "direct larval counts". Label the remaining three tubes (one top, middle, and bottom) as "DNA".

   2. Pour the sample onto the 35-u screen and use the squirt bottle to rinse the contents down to the bottom of the filter.

   3. Rinse the sample into a 50-mL centrifuge tube. Ensure that the volume does not exceed 50% of the total tube volume.

   4. Add 95% EtOH. Close tube, invert 6 times gently. Store at 4°C.

   5. Invert tubes again after 24 h.

4. Laboratory analysis - DNA extraction

a. Materials
   - Hybridization or drying oven
   - Centrifuge with bucket rotor and microcentrifuge rotor
   - QiagenDNEasy kit (Qiagen part # 69506) for Blood and Tissue
   - 95% Ethanol (EtOH) Pipettes and tips

b. Methods
   1. Site preparation

      - Turn hybridization oven on and allow warming to 37°C.

   2. Follow directions of the DNAeasy Kit: All buffer names are used as listed in the extraction kit manual.

      1. Centrifuge50 ml samples @ 4,000 RPM for 5 min
      2. Decant as much as possible of the EtOH / NaCl preservative w/o resuspending the pellet
      3. Warm the sample to 37°C for 3 hours in a hybridization oven to dry off remaining EtOH

4. Add 180 µl Buffer ATL solution to each sample
5. Vortex on a laboratory microtube shaker (such as Vortex-genie) vigorously for 5-10 seconds or sufficiently long to ensure the pellet is resuspended.
6. Add 20 µl proteinase K (from DNeasy kit, (>600 mAU/ml, solution; Qiagen part # 19131 or #19133) solution to each sampleVortexvigorously 5-10 secondsto ensure pellet is resuspended
7. Bake in hybridization oven for 2-3 hours (56°C), vortexing every hour or until pellet is dissolved and solution is clear
8. Add 400 µl Buffer AL solution to each sample
9. Vortex 5-10 seconds to ensure mixing
10. Incubate at 56°C for 10 min.
11. Add 400 µl 95% ETOH to each sample.
12. Vortex 5-10 seconds
13. Open a sealed DNeasy Mini spin column (filter) in 2 ml collection tube per station.
14. Label lid with sample number. The samples will be transferred multiple times, so proper labeling is essential.
15. Pipette the 0.75 ml of mixture from each 1.5 ml tube into the 2 ml filter on top of a collection tube. Save the tip in the 1.5 ml tube.
16. Centrifuge (hinge side faces center of centrifuge rotor) for 2 minutes at 8000 rpm (6000 x g).
17. Discard flow-through
18. Pipette the remaining 0.75 ml of mixture from each 1.5 ml tube into the 2 ml filter on top of a collection tube.
19. Centrifuge (hinge side faces center of centrifuge rotor) for 2 minutes at 8000 rpm.
20. Discard flow-through
21. Set up new set of 2 ml collection tubes.
22. Remove spin column and place each sample in a new 2 ml collection tube.
23. Discard bottom half of previous collection tube.
24. Add 500 µl Buffer AW1 solution to each sample.
25. Centrifuge for 2 minutes at 8000 rpm (6000 x g).
26. Set up new set of 2 ml collection tubes.
27. Remove spin column and place each sample in a new 2 ml collection tube.
28. Discard bottom half of previous collection tube.
29. Add 500 µl Buffer AW2 solution.
30. Centrifuge for 5 minutes at 14000 rpm (20,000 x g).
31. Set up pre-labeled 1.5 ml tubes.
32. Remove spin column and place each in a labeled 1.5 ml tube.
33. Discard 2 ml collection tube and flow-through.
34. Add 200 µl Buffer AE solution to each sample.
35. Incubate for 1 minute at room temperature.
36. Centrifuge for 2 minutes at 8000 rpm (6000 x g).
37. Remove filter and discard. Keep the 1.5 ml collection tube.
38. Store 50 ul of the DNA extract at 4°C and conduct QPCR within 96 hours. Store the remainder of the extract (~ 150 ul) at -80°C for archival purposes.

5. Laboratory Analysis - Quantitative PCR Reactions

a. Materials
- 2X Brilliant II SYBR Green QPCR Master Mix (part # 600828-1) or 2X Brilliant II QPCR Master Mix (part # 600804) with 0.167x SYBR Green dye (diluted from 10,000x stock; Invitrogen S-7567 as per manufacturer's instructions)
- StratageneMasterMix reference dye.
- For DWH samples use Agilent Technologies MX3000Por MX 3005P quantitative PCR machine: http://www.genomics.agilent.com/CollectionOverview.aspx?PageType=Application&SubPageType=ApplicationOverview&PageID=291
- Plates or strips &cover caps from stratagene(Agilent 401333 or 401428 & 401425)
- 96-well plates that are suitable for the make of the thermocycler
- Bovine serum albumin (BSA)
- Forward and Reverse *Crassostreavirginica*-specific PCR Primers

b. Methods
1. Prepare master mix for 98 reactions (or number desired per plate):

	Stock Solutions	final concentration	Single reaction	98 reactions
Master Mix	2x	1x	12.5 ul	1225 ul
Forward PCR Primer	25 uM	1 uM	1 ul	98 ul
Reverse PCR Primer	25 uM	1uM	1 ul	98 ul
BSA	2.0 mg/ml	0.52 mg/ml	6.5 ul	637 ul
H20			1 ul	98 ul
reference dye	25uM	1.0uM	1 ul	98 ul
Total			23 ul	2254 ul

- Vortex master mix, and spin briefly to collect mastermix.
- Keep mixture on ice until ready for use. Note that SYBR is sensitive to light, so mastermix must be kept covered.

2. Plate setup
   - Column A: larval dilution series (for example: 1, 2, 5, 10 25, 50 100, 1000 larvae)

- Column B: adult standard curve (10 x 10-fold dilutions)
- Each plate should include a positive (adult and larval curves) and negative control. Each plate will have 1 reaction supplied with 2 ul of the water used in all dilutions but no oyster DNA. A positive reaction in this reaction is indicative of contamination.
- Each sample should be replicated 2 times

- Place reaction plate onto a clean tray so that it does not touch the benchtop surface, or come directly in contact with ice. This prevents transfer of residue and dirt that add background fluorescence.
- Add 23 ulmastermix to each well, slowly, using the same pipet tip. Do not push air at the end of the pippeting motion. Draw each aliquot from just beneath the meniscus.
- Add 2 ultemplate (extracted DNA from the sample or standard).
- Cover plate with QPCR cap strips. Centrifuge for 2 min at 1200 RPM to collect the mixture. Vortexing is not needed because the sample will mix during the first incubation.

3. Thermocycling (QPCR) parameters

   Cover plate with film. Spin plate down for 2 min at 1200 RPM to collect reaction in the bottom of the plate.

   - 10 min, 95°C (initialization: DNA melt phase)
   - 65 cycles of (DNA amplification phase):
     - 30 s, 94°C (denaturation step)
     - 40 s, 58°C (annealing step)
       (monitor SYBR Fluorescence at the end of each cycle)
     - 45 s, 72°C (extension/elongation step)

   - 30 s, 72°C (final elongation)
   - Melt cycle
     - Cool sample to 58°C, 30s
     - Melt curve (warm to 95°C at ~ 0.1 °C / 10 s)
     - monitor SYBR Fluorescence at maximum rate available on QPCR machine used (~ 1 x each well / 10s on a 96-well plate, rate will be higher if fewer samples are run)

   - Cool to 25°C
   - Analysis
     - Samples with multiple peaks should be discarded
     - Samples that deviate +/- 2C from standards should be discarded
     - QPCR plates with positive reaction in the negative control well should be rerun

   - Store plate in refrigerator

4. Data acquisition

   Export output file that contains quantification info and melt curve temperatures.

   Include an excel spreadsheet for each plate that contains the NRDA sample names per coordinate, master mix recipe, time started, time finished.

6. Lab counts of total bivalve larvae (non-oyster specific).

DNA analysis confirms the presence of *C. virginica* but may not provide a quantification of larval concentrations. Thus, traditional counts of bivalve larvae should be performed (Figure A-2).

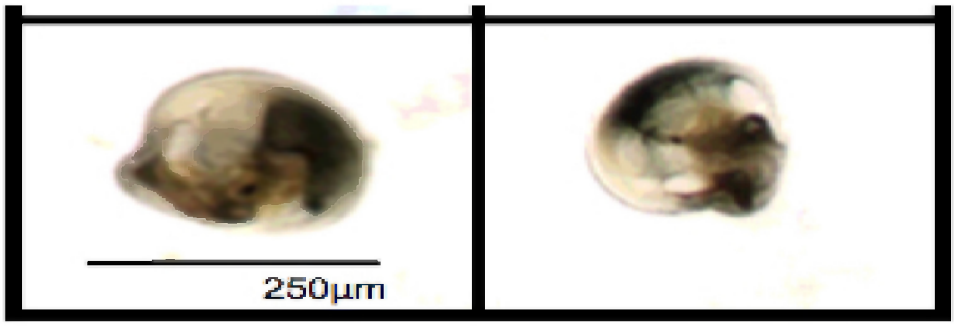


Figure A-2.Oyster (*Crassostreavirginica*) bivalve larvae under 20x magnification.

i. If possible, pipette the entire preserved and settled pellet into a 1 ml Sedgewick-Rafter cell (20x50 gridded slide) and count the entire sample. No extrapolations are necessary. If the entire sample fits onto the S-R cell, but larvae are extremely abundant (> 5 per grid cell after the first row), randomly select 4 of the 20 rows to count. The number of rows counted and the total number of larvae counted should be recorded. Because you will have counted 1/5 of the 20 rows, the estimate of the total number of larvae is as follows:

n * 5 = estimated total larvae / 1-Liter sample.

Return the contents to the 15 ml tube and maintain as evidence. DNA from these samples can be extracted if the DNA sample is lost but the sample is kept preserved.

ii. If the entire settled pellet does not fit into a 1 ml S-R counting slide, it must be subsampled.

a. Record the exact volume of sample.

b. Vortex the sample until the entire settled pellet appears to be in suspension.

c. Invert the sample several times then immediately withdraw a 1-ml sample. Ensure the pipette used has a wide enough opening that it does not clog if large particles, such as copepods or floc, are present. It is critical to pipette this 1-ml accurately.

d. count the sample on a S-R slide as described above. The estimate of the number of larvae per liter will be n / (1ml/total preserved sample)

e. repeat two additional times.

f. Average the three measurements and record the individual estimates and the average on a data sheet.

g. Return the contents to the 15 ml tube and maintain as evidence. DNA from these samples can be extracted if the DNA sample is lost but the sample is kept preserved.

iii. If the sample is very large it can be counted in a 10x10 gridded petri dish.

a. record the exact volume of the sample.

b. Stir the beaker vigorously prior to collecting the aliquot.

c. A Stimpel pipette should be used to collect a 5 or 10 ml aliquot of the sample. Record the exact volume of the subsample.

d. Place the aliquot in a clear petri dish.

e. Bivalve larvae in each section should be quantified and recorded on the data sheet. All sample should be examine under a dissecting scope at 50x magnification.

f. Extrapolate the subsample measurement to the entire volume of the samples. Divide total volume of sample analyzed in the lab by subsample volume and multiply subsample count by that number. For example 200ml/5 ml = 40, 40 * 6 bivalve veligers = 240 bivalves per 1L (original sample volume) or 240 bivalve larvae per L.

g. repeat the subsampling two additional times.

h. Average the three measurements and record the average on the data sheet.

i. Return the content of the petri dishes to the original sample and preserve as evidence. DNA from these samples can be extracted if the DNA sample is lost but the sample is kept preserved.

Field and Intake Materials Needed

- Nisken of LaMotte Water Sampler (fixed volume, remote trigger and 1 L capacity)
- 1-L sample bottles
- Waterproof labels
- 35 micron sieve (prepare extras in case of rips)
- 50-mL plastic centrifuge tubes
- 250-mL or 500-mL squirt bottles
- Field book
- 95% Ethanol solution. All chemicals must be reagent grade, and water should be filtered and deionized.
- Plastic transfer pipets
- COC forms for larval samples

C. SOP for Larval Settlement

Spat Sampling Methods

Spat settlement. Settlement plates made of cement board or other appropriate material will be placed at each subsample location within each site. Field teams will return at specified intervals (weather permitting) to attempt to locate and retrieve these boards to help evaluate settlement rates of spat.

1. Objectives

Quantify settlement and early survivorship (recruitment) of oyster spat.

2. Materials needed

- Concrete backer board or tiles
- Cable ties
- Ziploc bags (2 gallon size)
- Wire cutters
- Scissors
- Sharpie
- Weatherproof labels
- Crab traps with weight, line, and buoy

Setup:

- Standardized plates can be made from concrete backer board or tiles. Cut plates in 12 x 12 cm squares using a low speed saw. The inner 100 cm^2 will be used to enumerate settlers. Use only the inner 100 cm^2 so as to move away from an edge effect on the plate. Flow around the edge could be more turbulent than natural. It may increase or decrease settlement, but it could introduce variance in settlement unrelated to local conditions.

- Three settlement plates should be connected to a crab trap via cable ties (4 small ½ inch holes should be pre-drilled into the corners). (Figure A-3).

- Attach plates to the top of the cage spaced at least 30cm apart and rough side up. Attach a weight (approximately 5 lb.) via cable tie to the bottom of the trap for stability and attach a surface buoy. Rope should be long enough to account for wind and tidal induced changes in the water level, plus enough length to bring up on the vessel (rope length varies with area).

3. Field procedures

i. Label buoys with identifier that indicates the grid cell ID as well as the corner of the cell (i.e., NE or SW). Identifiers should be written directly on the buoy with a sharpie marker (do not affix a label with the sample ID numbers on duct tape).

ii. Two sets of three spat settlement plates may be placed at each site (cell) in the event that one set is lost during the deployment period.

iii. Record exact GPS position of deployment. Coordinates for plate deployment will be assigned and be calculated as the center point on the line between the northeast corner and centroid of the cell and the center point on the line between the southwest corner and the centroid of the cell. Crab traps will be deployed at these coordinated when possible.

iv. Depth should be checked either with the vessel's depth finder or by lowering a pole or rope over the side. Make sure the amount of rope attached to the pots is appropriate for the site before deploying the pot. 5-10' of rope beyond the depth is ideal.

v. Remove and replace plates every 21 days (+/- 2 days). If the schedule needs to be adjusted, plates should preferentially be retrieved earlier than scheduled (e.g., approximately two weeks), if weather conditions and personnel availability allow.

vi. Deploy plates on a crab trap near oyster substrate in a horizontal position. Where the water is shallow enough and the substrate soft enough, a 10' pvc pole may be planted very near the pot with the GCID and corner (i.e., NE or SW) marked on the pole.

vii. Retrieve pots and photograph with plates still attached.

viii. If during retrieval and replacement of settlement plates one trap is missing, do not deploy a replacement trap. If both traps are missing, deploy one replacement trap at either of the coordinates assigned for trap deployment. A trap found exposed during low tide, should be sampled and relocated and at traps located away from their initial deployment site, samples are to be collected and the trap returned to the original coordinates.

ix. Individually bag and label each retrieved plate; put all three plates into one bag with the sample ID and sample time. Each pot (3 plates bagged individually and then collectively) represents one sample.

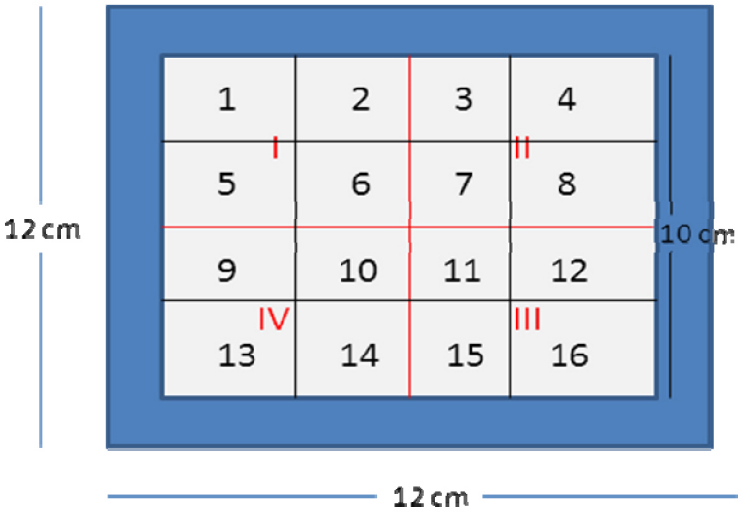
x. Store retrieved plates on ice and take to the intake laboratory. The surface and bottom of the plate should be marked on the plate by etching a B on the bottom side of the plate (side on the trap) with a screw driver or scraping tool. Do not mark the surface side.

4. Lab procedures

i. Freeze settlement plates until the plates are analyzed.

ii. Oysters on plates should be enumerated under 10x magnification and both live spats and spat scar (predated spat) should be enumerated.

iii. The top (surface exposed) of each settlement plate will be examined under a dissecting microscope at 10X magnification. The center area enclosed by a 10 cm x 10 cm frame will be examined for counts. The plates encompass a 12 x 12 cm area and the edges are not examined to minimize the influence of handling damage and hydrodynamic artifacts associated with the edge. For oyster spat, the entire inner 100 cm^2 area is examined and all live oyster spats and recently dead spats (denoted by scars) are enumerated. Other encrusting animals may be enumerated, or the plates may be archived for potential future enumeration of those other encrusting animals. If other encrusting animals are enumerated (e.g., barnacles and serpulid polychaetes), a subsample is randomly chosen and enumerated. Random selection occurs via a gridded, clear plexiglass overlay placed over the 100 cm^2 inner plate area. If non-oyster encrusting animals are estimated (visually) as >50 individuals a cell chosen to represent ¼ of the plate is enumerated for non-oyster encrusting animals. If non-oyster encrusting animals are estimated (visually) as >100 individuals, a grid representing 1/16 of the plate area is chosen randomly. Random selection occurs by assigning a number to each major grid and using an excel spreadsheet of random numbers from 1 to 4 or 1 to 16.



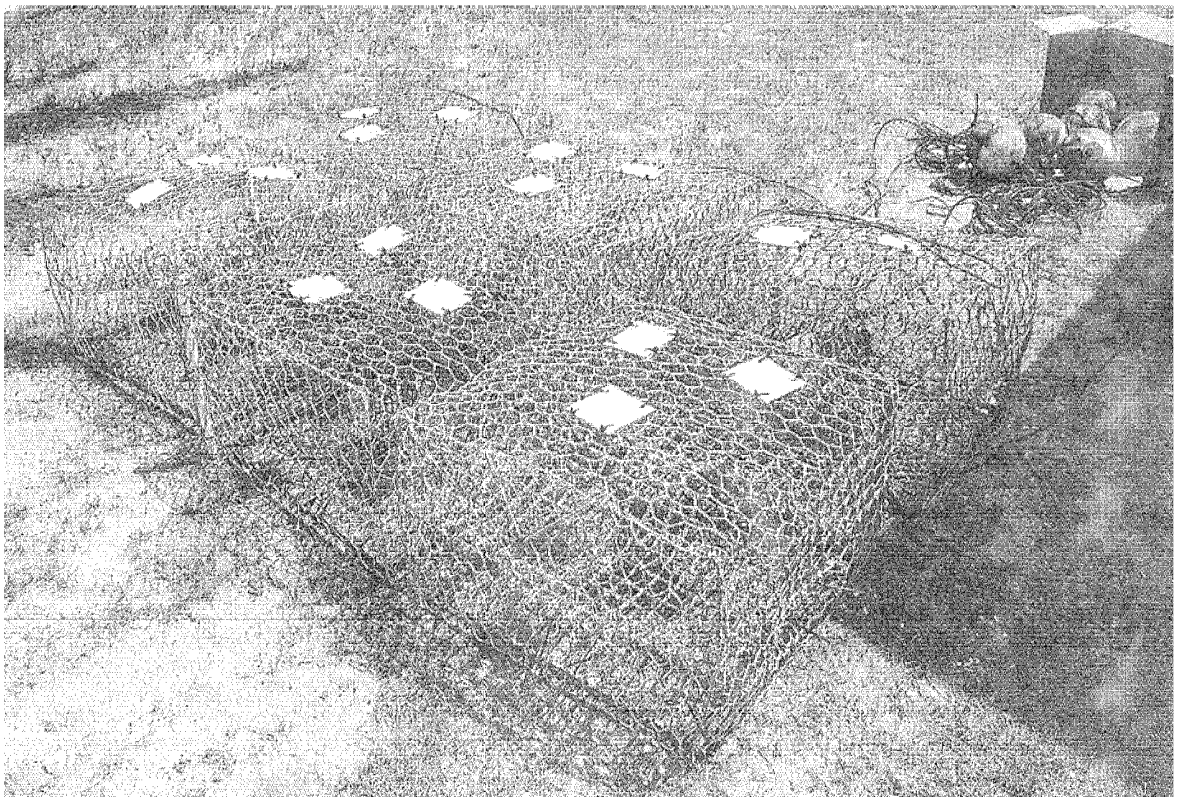


Figure A-3. Settlement plates attached to crab pot or trap. Photo courtesy of Jason Herrmann, AMRD.

D. SOP for Tissue Collection for Contaminant, Gonadal Condition, and Oyster Disease Analyses

1. Sampling Objectives

    a. Collection of oysters to document extent and duration of the area exposed to the spilled material. Bivalves uptake oil quickly, depurate them slowly, and can be used as "composite" samplers.

    b. Collection of oysters to determine the reproductive condition of oysters at each sampling site. These data can then be compared with larval supply and settlement data to determine potential impact of oil contamination on recruitment of oysters.

    c. Collection of oysters for oyster disease analysis.

    d. To maintain the integrity the sample(s) during sampling, transport, and storage.

2. Sample Size and pre-sampling activity

    a. 20 g wet weight for contaminant analysis (composite of ~4-6 individual market-sized organisms). Teams should collect 6 market-sized organisms if possible to ensure sufficient sample.

    b. At least 10 market-sized oysters for gonadal condition analysis. These same oysters will be used for oyster disease analysis.

    c. Clean dredges, knifes, etc. between samples. If no oil is visible wash in ambient water. If the equipment was obviously contaminated, scrub with Alconox solution. Collect rinsate for proper disposal.

3. Take relevant photos at all sites, including a picture of the dredge once it is full including overall contents and visual appearance of size/condition of oysters/shells in dredge.

4. Dredge Sampling Locations

    a. Up to eight randomly generated contact points will be used to determine dredge sampling locations. These contact points will be generated as a random sample of points from transect segments identified in the mapping exercise to contain Class III bottom surface.

    b. Field teams should begin dredging at the first contact point on the list and proceed to collect three dredge replicates (described below). If 20 market-sized oysters (or their equivalent –see below) have not been collected by this point, field teams may

continue to dredge at the remaining contact points until 20 market-sized oysters (or equivalent) have been collected or until two hours have elapsed, whichever comes first.

i. If market-sized oysters are not available, the equivalent wet weight tissue from seed-sized oysters can be used for contaminant analysis. The ratio of seed to market-sized oysters is 1.5:1 (i.e., if 6 market-sized oysters are collected, 6 seed-sized oysters should be collected to reach the total goal of 10 market-sized or equivalent).

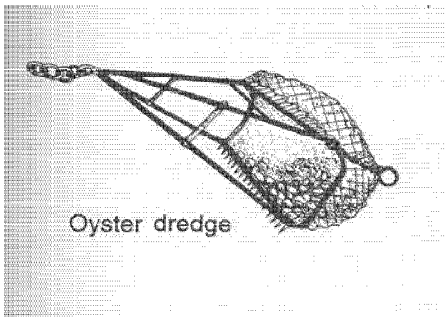
5. Sample Collection Methods

   a. Dredge harvesting using a 24 inch wide oyster dredge may be used to collect resource:
      i. Deploy dredge from the beam or stern of the vessel.
      ii. Record exact start and stop positions using a GPS. Start location is the point at which the dredge enters the water. Stop is the point at which the vessel stops moving in a forward direction (i.e., the stop point will be marked before the dredge is brought onboard).
      iii. Drag dredge across the surface of the substrate for 3 minutes at 2 knots in a circular pattern.
      iv. Conduct three (3) dredge pull replicates at the first three contact points provided to the field team (i.e., 1 pull each per contact point)..
      v. Additional dredge pulls beyond the initial three may be performed if needed to obtain the target number of oysters; however, these pulls are not considered replicates, and the oysters from the extra dredges do not need to be enumerated by size category. Additional dredges may be performed until the required number of live market size oysters (~20) is collected (or until 2 hours have passed).
      vi. If enumeration is to take place at the intake lab, place animals from each individual dredge in a burlap sack.
         1. Gently agitate the sack to remove excessive mud or debris.
         2. Close sack.
         3. Place the burlap sack in plastic bag.
         4. Samples should be tagged with an external (flagging tape with permanent marker) and internal tag (flagging tape with permanent marker) that prominently denotes sample code.
         5. The sample code should be constructed of the location ID, date, matrix, sample team leader code, and sample number along with information regarding sample type (for details, see the Oyster Sample ID Naming Convention, Appendix B).
         6. Hold animals on ice until delivered to intake team.

6. Enumeration of Oysters and Sample Preparation

i. Enumeration of oysters and preparation of contaminant and gonad and disease samples may be conducted by field staff trained by DISL representatives or at the intake lab by DISL staff. For efficiency in field efforts, it is preferred that samples be enumerated at the intake lab.
ii. All live oysters and recently dead oysters from each replicate are to be enumerated into the following size categories:
    1. spat (less than 1 inch [25 mm] shell height),
    2. seed or juvenile oysters (between 1 and 3 inches [25 – 75 mm]),
    3. market size or "legal" oysters (> 3 inches [75 mm] shell height].

    Separate enumerations should be made of the live and recently dead oysters. "Recently dead" oysters are defined as articulated (i.e., hinged) shells ("boxes") with no evidence of fouling on the inside of the shell (i.e., no evidence of barnacles or other organisms attached to the inside of the shell).
iii. Retain the first twenty (20) live, market-size oysters encountered as samples for later analysis - 6 oysters for tissue contaminant analysis and 14 for disease/gonad analysis. The remainder of each dredge's materials can be retained and kept separate while other contact points are enumerated, if it is necessary for ensuring that the equivalent of 20 market size oysters are sampled.
iv. In the event that an insufficient quantity of market size oysters (i.e., less than 3") can be collected for analysis, keep up to 30 oysters that are seed size (1 – 3 inches) by arranging the oysters less than 3" by decreasing size, then taking the 30 largest oysters and noting how many were retained from each dredge.
v. If after dredging for the prescribed period less than 4 market-size oysters or equivalent are collected, then all oysters should go for contaminant analyses. If less than 20 but greater than 4 market-sized oysters or equivalent are collected, designate the first 4 market-size oysters or equivalent for contaminant analysis, and then randomly assign each additional available oyster to contaminant or gonad/disease by flipping a coin.
vi. Preparation of oysters for contaminant analysis (6 oysters): Wearing clean latex or nitrile gloves Wrap each oyster individually in aluminum foil then place all wrapped oysters in a 2-gallon Ziploc bag. Close bag.
vii. Preparation of oysters for disease gonad analysis (14 oysters): Place wrapped oysters in a 2-gallon Ziploc bag. Close bag.
viii. Samples should be tagged with an external (flagging tape with permanent marker) and internal flagging tape tag that prominently denotes sample code.
ix. The sample code should be constructed of the location ID, date, matrix, unique sampler ID, and sample number along with information regarding sample type (for details, see the Oyster Sample ID Naming Convention, Appendix B).
x. Hold animals on ice until delivered to intake team.



b. Wear nitrile or other non-contaminating gloves and change gloves after each sample to avoid cross-contamination. Record observations of any external evidence of contamination.
c. Shellfish should not be opened in the field to minimize the risk of contamination.
d. Avoid sources of contamination such as exhaust fumes and engine cooling systems on vessels. Work upwind of any exhausts. Segregate dirty/clean areas. Lay out clean substrates to work on and replace frequently. Take precautions so as not to introduce cross-contamination from oil on boots and shovels.
e. Use packing material around sample containers to prevent breakage during handling and shipping.
f. When accounting for other species present in the dredge on the field form (Transition Plan Oyster Field Forms Dredge Sampling) it is important to document the presence of oyster drills in the sample; blue crabs and mud crabs will not be recorded.

7. Preservation/Holding Times

   a. Immediately place all samples in cooler and keep at 4°C. Freeze as soon as possible (if contaminant sample; do not freeze gonad/disease samples).

   b. Live contaminant oyster samples not designated for analysis at time of collection will be frozen at -20° C until such time as they are designated for analysis, up to one year (per recommended holding time).

   c. Please see the Analytical Quality Assurance Plan for the MS Canyon 252 (Deepwater Horizon) Natural Resource Damage Assessment (QAP) for further details on storage and holding times.

8. Labeling, Documentation, and Other Considerations.

   a. On the FTP site, the NRDA Field Sampling Checklist generically summarizes pre- and post-field sampling tasks.

b. Prepare sample labels as presented in NRDA Data Management Protocol for Field Sampling. If using jars, record the sample number on both the label and lid. IDs on sample labels must be complete and exactly identical to IDs on the chain of custody. Jar labels receive a protective layer of clear tape wrapped around the entire circumference of the container to secure the label and protect the writing.

c. See the event-specific protocol documents for shipping to designated labs (NRDA Sample Shipping Instructions) and for chain of custody and sampling documentation instructions (NRDA Data Management Protocol for Field Sampling). Tissue sampling log sheets typically record sample number; date/time, location, GPS coordinates, species and tissue type.

d. Documentation is critical; all field notebooks should be dated, signed, and preserved. If crossing out or correcting any entries, date and initial when making the changes. Original records will be gathered and archived.

e. Record the presence of oil, weather conditions, etc. in field notes. Record GPS coordinates for each sample. Any oil slicks should be immediately reported to the NRDA Field Operations office along with coordinates and a detailed description of the size and consistency of the sheen.

f. Take relevant photographs of the sampling locations and sample collection itself if possible. Make sure each photograph or series can later be associated with the corresponding sampling location GPS (see NRDA Field Photography Guidance). Do not delete, open or alter any photos.

g. All sampling, COC, shipping, GPS and photo files are submitted to [redacted] Sampling hotline: [redacted].

9. Analytical Methods for Contaminant Analysis

The collected tissue samples should be analyzed in accordance with the MS Canyon 252 QAP. Specific suites of analytes to be measured include:

i. Polynuclear Aromatic Hydrocarbons (PAH), including both standard and alkylated PAHs. Sterane/triterpane biomarkers will be analyzed and quantified as needed. See full list in Table 1.1a of the QAP, which also specifies the target method detection limits.

ii. Lipid content. Lipid content is defined as the percent of sample tissue extracted and remaining after solvent evaporation. It is used to normalize organic contaminants in tissues, to aid in spatial and temporal comparisons among samples.

Equipment List

i. Shovels and/or trowel
ii. Knife
iii. Dredges
iv. Tongs
v. Gloves (nitrile and knit Kevlar)
vi. Screen (for sieving out sediment)
vii. Aluminum foil
viii. Certified-clean glass jars
ix. Ziploc bags
x. Cooler and ice
xi. Marker pen
xii. Waterproof sample labels
xiii. Clear tape
xiv. Burlap sacks
xv. Contractor grade garbage bags
xvi. Flagging tape
xvii. 5-gallon buckets

PLEASE NOTE: Avoid sources of contamination such as exhaust fumes and engine cooling systems on vessels. Work up-wind of any exhausts. Segregate dirty/clean areas. Lay out clean substrates to work on and replace frequently.

E. SOP for Sediment Collection for Contaminant Analysis

1. OBJECTIVE

Collect 375 mL of surface sediment (0-2 cm) including the flocculent material residing at the sediment water interface at predetermined locations. Collect 375 mL of subsurface sediment (2-4 cm) for archive. Collect four individual sediment samples per grid cell. Field teams will be provided with up to eight randomly generated contact points per grid cell. These contact points will be generated as a random sample of points from transect segments identified in the mapping exercise.

2. FIELD EQUIPMENT

Each crew should be deployed with multiple sampling devices to rapidly determine the general sediment type and apply the most appropriate sampling method to the field conditions. The basic sampling equipment should include the following elements.

Boat

The near-shore sediment sample collection methods will be deployed off small vessels (generally 12 to 40 ft fishing boats) with no winch, lifts, moon pools, or special anchors. However, these devices can be used, if available.

Weighted Tape Measure

The boats are typically equipped with an acoustic depth finder that generally has a precision of approximately +/- 1 ft. However, the depth finder may not be located where the sample is to be collected. It is recommended that the field team confirm the water depth with a precision closer to +/- 1 inch to help the sample operator gently lay the sampler on the sediment surface. The depth should be confirmed with an open reel tape measure with a weighted end (lead line) and saltwater-tolerant construction. For extremely fine sediments with a heavy flocculent layer, a small disc (approximately 6 inches in diameter) can be placed at the weighted end of the line to better identify the depth of the sediment-water interface. In waves or swells, the average water depth is estimated at half the distance between the top and bottom of a wave. Other comparable methods may be used as needed.

Samplers

The crews should be equipped with at least one grab sampler (e.g., van Veen) and one core sampler (e.g., piston corer). The diver corers are used as a backup in the event that the first two options are not deemed successful. Table A-1 summarizes the features of the recommended samplers:

Table A-1. Specifications for Recommended Sampling Equipment.

Sampler	Dimensions	Dimensions	Surface Area	Maximum Surface Sediment Volume	Estimated Loss During Handling	Estimated Surface Sediment Volume per Attempt	Estimated Number of Attempts for 375 mL Sample
	In	cm	cm^2	cm^3 or mL	%	mL	
van Veen	8" x 6"	20.3 x 15.2	308	616	10	554	1
Ponar	6" x 6"	15.2 x 15.2	232	464	10	418	1
Ekman grab	6" x 6"	15.2 x 15.2	232	464	10	418	1
Piston Core	3" diameter	7.62 diameter	45.6	91	0	91	4 to 5
Piston or Diver Core	4" diameter	10.2 diameter	81.1	162	0	162	2 to 3

van Veen Grab Sampler

For most substrate types, the van Veen sampler may be the preferred sampler. The van Veen grab sampler is a "clam-shell" device with opposing lever arms that close after the device contacts the sediment surface and the operator lifts the lever arms towards the boat. For hand deployment, a small van Veen grab is preferred, approximately 8" x 6" x 6" (20.3 cm x 15.2 cm x 15.2 cm). It is important to note the sampler dimensions to appropriately determine the area sampled. A grab that is 8" X 6" will sample an area of 308 cm^2. One grab should produce an adequate quantity of sample for laboratory testing and archive. It is recommended that the van Veen have screened doors on the top of the device to facilitate the movement of water through the device and reduce the bow wave that might otherwise push the flocculent material away from the sampler as it reaches the sediment water interface. In addition, the sampler should have a compound bucket profile that allows for the full area of the sampler to be sampled to the target depth. The device can include a stabilizing cage to assure perpendicular penetration. When needed, the device should accommodate the attachment of weights to facilitate the penetration of hard sediments. The location of these weights should not adversely affect the even closure of the clam-shell doors. The device should possess overlapping edges to retain water when closed. Finally, it is recommended that the rope on which the sampler is deployed to the sediment surface be marked (e.g., Sharpie or electrical tape) at every foot interval above the open mouth of the sampler so the sampling team can assure its gentle placement on the sediment water interface. The sampling personnel should handle the grab sampler carefully to avoid the accidental closure of the device on fingers, hands, or feet.

Ekman or Birge-Ekman

The Ekman grab sampler may be the preferred sampler for sediments with high amounts of flocculent material overlying the sediment surface. The van Veen sampler can work in these soft sediments, but care must be taken to avoid over-penetration. The Ekman sampler has spring-loaded jaws mounted on pivot points on the sides of a metal box. The dimensions are typically 6" X 6" X 6". The Ekman can be deployed by rope with a messenger, but the performance is improved by mounting it on a pole that allows the sampler to push the grab into the substrate. The jaws are activated from the surface by a push-knob at the top of the pole. Flaps on the top of the sampler open on descent to prevent a bow wave and close on ascent to reduce erosion or mixing. The pole may limit the water depths in which this sampler may be used. For the purposes of this survey, the Ekman should only be used at shallow sites with soft sediment. The sampling personnel should handle the grab sampler carefully to avoid the accidental closure of the device on fingers, hands, or feet.

Ponar Sampler

The Ponar grab sampler is a clam shell device with two opposing jaws with scissor arms that allow the two halves of the Ponar sampler to close after the sampler contacts and is subsequently lifted from the bottom. The Ponar sampler should be considered a backup device in the event that the van Veen sampler is not available, because the automated trigger mechanism is not as precise and accurate as the van Veen sampler. Ponar samplers are well suited for the collection of medium to moderately hard surface sediments. They are manufactured in different sizes to address project specific objectives. The preferred dimensions are 6" X 6" X 6". Most Ponar samplers used to support the Oyster TWG programs are self-tripping, with a spring-loaded pin that releases when the sampler makes impact with the bottom. When closed, the jaws of the grab overlap preventing sediment loss. The top contains a screen that allows water to pass through the device and assure an

even vertical decent and limits the creation of a bow wave as the sampler is lowered to the bottom. Rubber flaps cover the screens when the clam shell is closed and prevent sample loss when the device is lifted to the boat. The penetration of the sediment is largely controlled by the weight of the device and the force of the lever arms. Finally, it is recommended that the rope on which the sampler is deployed to the sediment surface be marked (e.g., Sharpie or electrical tape) at every foot interval so the sampling team can assure its gentle placement on the sediment water interface. The sampling personnel should handle the grab sampler carefully to avoid the accidental closure of the device on fingers, hands, or feet.

Modified Piston Core Sampler

The piston core can be used to confirm the sediment structure observed in the van Veen sampler (e.g., how thick is the flocculent material at the sediment water interface) or to collect subsurface sediments. A piston corer consists of a plastic core tube liner mounted on an aluminum head, and is fitted with extension rods that are sized to reach from the operator to the sediment surface. A piston slides within the core barrel, providing suction within the core tube and preventing sediment from flowing out the bottom of the sampler. The position of the piston is held at a fixed height above the sediment surface by a rope that extends to the operator. Alternatively a one-way flapper or ball valve can be used to retain the sediment sample, removing the piston and line from the apparatus, better preserving the surface layers and simplifying the sampling procedure. The use of the one-way flapper valve in place of the piston is the recommended sampler for this program.

The core liners are typically made of stiff clear plastic (e.g., polycarbonate). The volume of surface sediment is controlled by diameter of the core liner. The recommended piston core liner for silts and clays with organic matter is 10.16 cm diameter x 61 cm height (4" x 24") and the area sampled is 81 cm^2. The recommended piston core liner for flowable sands is 7.62 cm diameter x 61 cm height (3"x24") and the area sampled is 46 m^2. The liner thickness should be 0.16 cm (1/16"). The piston core head should be modified to accommodate either 3" or 4" core liners in the field. Multiple cores are required for the production of large sample sizes. Five replicates of the 3" diameter core liner and three replicates of the 4" core liner should produce the amount of sediment required for laboratory testing (375 mL).

To collect a sample, the core tube is placed on the surface of the sediment, the rope fixed to a stationary object, and force (hand or hammer) applied to the top of the corer to drive it into the sediment. It is recommended that a temporary mark (e.g., electrical tape) be placed on the extension pole indicating the water depth and targeted penetration depth. Once the desired depth is reached, the piston line is held tight against the extension rod and pulled up with the core. Care should be taken not to allow the piston line to move relative to the core tube. If a flapper or ball valve is used, the core is simply pulled up from the bottom and recovered on the vessel.

Diver Push Core Sampler

The Diver push corers are plastic tubes that SCUBA or surface assisted divers push into the sediment manually. They work best in soft to moderately hard sediment. The core liners are typically made of stiff, non-reactive plastic (e.g., polycarbonate). Once inserted into the sediment to a depth of approximately six inches, a polyethylene cap is placed on the top of the core. After a small hole is dug to the side of the corer, the diver places a second polyethylene cap on the bottom of the corer and returns to the boat with the core in its original vertical position. Alternatively, the diver may slide his/her hand under the core while it is extracted from the sediment. The volume of surface sediment is controlled by the diameter of the core. The recommended diver core liner is 10.16 cm

diameter x 61 cm height (4" x 24") and the area sampled is 81 cm^2. The liner thickness should be 0.16 cm (1/16"). Multiple cores are required for the production of large sample sizes. Three replicates of the 4" core liner should produce the amount of sediment required for laboratory testing (375 mL). At every sampling location, the diver should descend with a basket capable of holding the cores in an upright orientation. The basket should contain 4 core liners and 8 core caps. One of these core liners is a backup in the event that the diver suspects problems with one of the initial 3 replicate cores.

3. TIERED APPROACH FOR SAMPLE COLLECTION

The field team will use a tiered approach for determining the most appropriate method for sample collection. For most substrate types the van Veen should be the first sampler attempted. Upon recovery, the operator will determine if the sample is acceptable. If an acceptable sample cannot be collected, either the Ekman (in very soft sediment) or a core sample should be attempted.

Acceptable Sample Criteria

An acceptable sediment sample consists of at least 5 cm (>2") with an intact surface layer. Acceptable samples should not have sediment in direct contact with the doors and should not have sediment pushing through the door screens. If the overlying water drains out of the sampler, it should remain clear and should not significantly erode channels in the recovered sediment. A flocculent layer should appear on the surface when one is know to exist.

A sediment sample should be considered unacceptable if the surface layer contacted the top of the sampler or exhibited obvious features of mixing (e.g., no difference between the surface and subsurface when differences were known to exist). The absence of flocculent material in the van Veen sample should be confirmed by collecting a piston core. The van Veen sample should be recollected if the piston core sample exhibits significantly more flocculent material than the van Veen sample. An acceptable quantity of sediment should be collected within approximately 10 attempts. If this is not possible, the samplers should assess the samples to determine the reasons for poor performance and consider either an alternative location or alternative sampler.

3.1 Sample Collection with the van Veen Grab Sampler:

1. Upon arrival at the station, the field team should begin by confirming the water depth with a weighted tape measure and record station coordinates.
2. Decontaminate the van Veen grab sampler using a three-step field decontamination process. Ensure that all surfaces are free of visible sediment. Thoroughly wash all surfaces with soapy water and a bristle brush and then rinse with site water. Follow the site water rinse with a rinse on all surfaces with deionized water. If site waters are not considered suitable for a site water rinse, deionized water may be substituted.
3. Ready the sampler for deployment. Ensure that the screened doors are locked shut. Set the sampler by lifting up on the chain at the shackle. Clip the ring in the pelican hook. As the grab is lifted, the jaws of the grab will open.

4. Lower the grab steadily to the sediment surface. It is important to lower the sampler slowly to the bottom, at a rate no greater than 1 ft/sec to prevent a bow wave. If the site is in deeper waters, the sampler may be lowered more rapidly until approximately 10 ft. above the bottom. The calibrated 1-foot marks on the rope will help the sampling personnel assure a gentle placement.
5. To collect a sample, allow the line to go slack once the sampler is on the bottom. Then, pull up slowly on the line, lifting the grab sampler causing the buckets to close. In firm substrate types, it is good to allow the sampler to "rest" on the bottom for approximately 30 seconds to allow it to penetrate the bottom. In softer substrate, minimize the contact time to prevent over-penetration.
6. As the sampler is retrieved to the sampling vessel, take care not to accidentally open the grab. The sampler can be hoisted by the chains or by one of the arms. Once retrieved, open the screened doors to inspect the sample.
7. If the sample is considered acceptable using the criteria listed above, remove the overlying water using a siphon or other similar means, taking care not to remove the flocculent layer. The unused overlying water can be returned to the sampling area. Using a clean stainless steel spoon, collect the top 2 cm of sample taking care not to collect sediment in direct contact with the sampler walls.
8. Using a clean stainless steel spoon, collect the next deeper sediment layer (2 - 4 cm) for archive. Take care not to collect sediment in direct contact with the sampler walls. Take precautions, like removing extra water or sediment to minimize the amount of sediment from the 0-2 cm interval that mixes with the 2 - 4 cm interval.
9. Process samples in a manner consistent with the instructions in Section 4.
10. If the sample is not acceptable, open the jaws of the grab, rinse clean of sediment and redeploy. If the sample has washed out, check the jaws for any debris preventing closure.

3.2 Sediment Collection with the Birge-Ekman Grab Sampler:

1. Upon arrival at the station, the field team should begin by confirming the water depth with a weighted tape measure and record station coordinates. Ensure that the water depths are within the capabilities for the Ekman sampler (generally $\leq$4 ft. with a standard pole).
2. Decontaminate the Ekman grab sampler using a three-step field decontamination process. Ensure that all surfaces are free of visible sediment. Thoroughly wash all surfaces with soapy water and a bristle brush and then rinse with site water. Follow the site water rinse with a rinse on all surfaces with deionized water. If site waters are not considered suitable for a site water rinse, deionized water may be substituted.
3. Ready the sampler for deployment. Set the sampler by pulling the jaws open and place the cable loops over the small pin near the triggering mechanism. Take care to keep fingers out of the closing path of the jaws.
4. Lower the grab to the sediment surface. Based on the lead line measurement, use depth marks on the sampler pole to determine when the sampler is at the sediment surface. In firm sediments, the sampler should push the grab into the sediment to a depth of approximately 4

to 5 inches. The Ekman grab is 6 inches in depth and care should be taken to avoid over-penetration. In soft sediment, little force will be required to push the sampler to the desired depth. In very fine sediment with an extensive flocculent layer, there may be little to no resistance as the grab is lowered into the substrate. In such cases, it is important to use the lead-line measurement to determine the maximum depth to lower the grab.

5. Once the grab sampler is in position, push the trigger at the top of the pole.
6. Retrieve the sample by pulling the pole slowly and steadily to the surface. Care should be taken keep the sample upright and keep the pole in the vertical position. As the sampler is retrieved to the sampling vessel, take care not to accidentally open the grab. Once retrieved, open the doors to inspect the sample.
7. If the sample is considered acceptable using the criteria listed above, remove the overlying water using a siphon or other similar means, taking care not to remove the flocculent layer. Using a clean stainless steel spoon, collect the top 2 cm of sample taking care not to collect sediment in direct contact with the sampler walls.
8. Using a clean stainless steel spoon, collect the next deeper sediment layer (2 - 4 cm) for archive. Take care not to collect sediment in direct contact with the sampler walls. Take precautions, like removing extra water or sediment to minimize the amount of sediment from the 0-2 cm interval that mixes with the 2 - 4 cm interval.
9. Process samples in a manner consistent with the instructions in Section 4.
10. If the sample is not acceptable, open the jaws of the grab, rinse clean of sediment and redeploy. If the sample has washed out, check the jaws for any debris preventing closure.

3.3 Sediment Collection with the Ponar Grab Sampler:

1. Upon arrival at the station, the field team should begin by confirming the water depth with a weighted tape measure and record the depth along with station coordinates. In instances of strong wind or current, the boat should be anchored to maintain location and for ease of sampling.
2. Decontaminate the Ponar grab sampler using a three-step field decontamination process. Lock the grab in the open position with the safety pin. Ensure that all surfaces are free of visible sediment. Thoroughly wash all surfaces with soapy water and a bristle brush and then rinse three times with site water. Follow the site water rinse with a rinse on all surfaces with deionized water. If site waters are not considered suitable for a site water rinse, deionized water may be substituted.
3. Ready the sampler for deployment. Ensure that the screened doors are locked shut. Set the sampler by replacing the safety pin with the spring-loaded pin, and applying vertical tension on the arms of the grab to keep the pin in place.
4. Lower the grab steadily to the sediment surface. It is important to lower the sampler slowly to the bottom, at a rate no greater than 1 ft/sec to prevent a bow wave. If the site is in deeper waters, the sampler may be lowered more rapidly until approximately 10 ft. above the bottom. Uneven lowering or sudden changes in tension on the line can cause the spring-

loaded pin to prematurely fire. The calibrated 1-foot marks on the rope will help the sampling personnel assure a gentle placement.

5. To collect a sample, allow the line to go slack once the sampler is on the bottom. It may be necessary to gently shake the line, moving the arms of the grab slightly and reducing the pressure on the pin causing it to trigger. Then pull up slowly on the line, lifting the grab sampler and causing the buckets to close. In firm substrate types, it is good to allow the sampler to "rest" on the bottom for approximately 30 seconds to allow it to penetrate the bottom. In softer substrate, minimize the contact time to prevent over-penetration.
6. As the sampler is retrieved to the sampling vessel, take care not to accidentally open the grab. The sampler can be hoisted by the line or by squeezing the arms together above the hinge on the arms. Once retrieved, open the screened doors to inspect the sample.
7. If the sample is considered acceptable using the criteria listed above, remove the overlying water using a siphon or other similar means, taking care not to remove the flocculent layer. Using a clean stainless steel spoon, collect the top 2 cm of sample taking care not to collect sediment in direct contact with the sampler walls.
8. Using a clean stainless steel spoon, collect the next deeper sediment (2 - 4 cm) for archive. Take care not to collect sediment in direct contact with the sampler walls. Take precautions, like removing extra water or sediment to minimize the amount of sediment from the 0-2 cm interval that mixes with the 2 - 4 cm interval.
9. Process samples in a manner consistent with the instructions in Section 4.
10. If the sample is not acceptable, open the jaws of the grab, rinse clean of sediment away from sampling site and redeploy. If the sample has washed out, check the jaws for any debris preventing closure.

3.4. Sediment Collection with the Piston-Core Sampler:

The piston core should be used if the van Veen or other grab samplers are not able to recover an acceptable sample after about ten attempts. A one-way valve is recommended in place of the rubber piston normally used. This will improve the ease of use and efficiency of the sampler. The valve is attached to the core head in a vertical position allowing air and water to exit as the core tube is pushed into the sediment and providing suction to hold the sediment in place as the tube is pulled upwards. Hard objects such as rocks, oysters, or shell-hash may impede penetration to target depth. Using a larger diameter tube may help avoid the interference of hard objects. Multiple attempts needed to obtain sufficient sediment quantities for analysis should be made, moving up-current from previous attempts, until sufficient volume is collected or it is determined that piston-core sampling is not feasible in the area. Four core samples should be collected if using a 4"-diameter core tube; a total of 8 core samples will be needed if using a 3"-diameter core tube. Core samples should be collected with 18" or 24" core tubes and should be pushed into the sediment 12" to 18" into the bottom, or to the point of resistance (when the core cannot be pushed any further). A hammer (e.g., mallet or slide hammer) can be used to drive the core further into sediment, however, care should be taken not to drive the core in to the point that it cannot be pulled out by hand.

It may be necessary to conduct diver-operated coring in these situations as sample location can be chosen to avoid hard objects.

The following table (Table A-2) can be used to determine when to use a 3" diameter versus 4" diameter core tube for sampling. Alternative configurations can be used if comparable or better sample integrity can be achieved.

Table A-2. Piston Core Configuration.

Sediment Type	Texture	Diameter (inches)	Target Length (inches)
Organic Silt	Soft	4	12 - 18
Silty Sand	Intermediate	4	18
Sand	Hard	3	12-18
Shell or Veg	Hard	3 or 4	12-24

The procedure for collecting a sample using the piston corer follows:

1. Upon arrival at the station, the field team should begin by confirming the water depth with a weighted tape measure and record the depth along with station coordinates. The type of sediment felt when measuring depth should be noted.
2. It is important to collect a core sample from a stable platform. In instances of strong wind or current the boat should be anchored to maintain location and for ease of sampling. The core tube must remain as close to vertical as possible to collect an acceptable sample.
3. Decontaminate core tubes and caps using a three-step field decontamination process. Ensure that all surfaces are free of visible sediment. Thoroughly wash all surfaces with soapy water and a bristle brush and then rinse three times with site water. Follow the site water rinse with a rinse on all surfaces with deionized water. If site waters are not considered suitable for a site water rinse, deionized water may be substituted.

   Alternatively, core tubes may be pre-cleaned by the analytical laboratory and wrapped in aluminum foil. In such cases one core tube should be used to collect all sediment at a given station.
4. The piston core head is a cylindrical piece of aluminum, which has a threaded hole at one end to receive the extension poles. The first extension pole should remain attached to the piston head. Additional poles may be added to accommodate the water depth at the station. It is recommended to mark the extension rods with one-foot increments.
5. Attach the core tube to the head using hose clamps and add extension rods as needed to the other end of the head. Lower the piston core sampler into the water allowing the core tube to fill with water. Air and water should come out of the one-way valve on the side of the piston head.
6. Gently lower the core tube until it contacts the sediment surface, being careful not to disturb the flocculent layer and keeping the tube and rods vertical. Note the location of the waters

surface on the side of the extension rod and note the mark 12" or 18" (depending on the desired length of core) above the water surface. Push the sampler straight down using hand force if possible or, if necessary, a small sledge or slide hammer, until the second mark is reached.

7. Gently pull the sampler straight up removing extension rods as needed. Place a cap on the bottom of the tube when it reaches the surface of the water. The tube can then be set on the boat and the hose clamps and head can be removed. In softer sediments, the cap will need to be placed over the bottom quickly to avoid the loss of sediment from the bottom. Some loss is acceptable if the top 5 cm (2") are primarily undisturbed.
8. Rinse the outside of the core tube with water, then measure and photograph the core.
9. If the sample is considered acceptable using the criteria listed above, remove the piston core head by releasing the hose clamps. Remove the overlying water using a siphon or other similar means, taking care not to remove the flocculent layer.
10. Using a clean stainless steel spoon, collect the top 2 cm of the sample. Depending on the length of the tube and the sediment core it may not be possible to reach the sediment surface with a spoon. If the sediment is out of reach, the core tube can be cut with a hack saw one inch above the sediment surface, or the sediment can be pushed up from the bottom using an extruder until the surface sediment is reachable with a spoon.
11. Repeat the previous procedure for the collection of the next deeper sediment layer (2-4 cm) for archive.
12. Process samples in a manner consistent with the instructions in Section 4.
13. If the sample is not acceptable, remove the tube from the head, rinse clean of sediment away from sampling site and redeploy.

3.5 Sediment Collection with Diver Operated Core

1. Upon arrival at the station, the field team should begin by confirming the water depth with a weighted tape measure and record the depth along with station coordinates. When divers are sampling from a boat, anchoring is highly recommended to maintain a stable station location and provide a safe working area for divers.
2. Four inch diameter core tubes are either pre-cleaned and wrapped in foil or cleaned in the field. Cores cleaned in the field should follow a three-step decontamination procedure. Thoroughly wash all surfaces with soapy water and a bristle brush and then rinse three times with site water. Follow the site water rinse with a rinse on all surfaces with deionized water. If site waters are not considered suitable for a site water rinse, deionized water may be substituted.
3. Divers should approach from down-current of the station taking care not to disturb surface sediments. For sites with fine sediments and flocculent layers, great care should be taken to approach the site slowly and using buoyancy to settle to the bottom without disturbing surface materials. Limited visibility may require marking a station with a buoy and divers descend the buoy line to the station.
4. Once at the bottom, the diver will locate appropriate substrate for sampling, avoiding areas that are not representative of the station, areas of substantial rock, or areas that exhibit thick,

dense shell hash. If water is turbid from the divers' approach, the sampler should wait until the suspended sediments near the sediment surface have settled and the water returned to its ambient condition.

5. Divers descend with four tubes and eight caps so that sufficient material can be collected in one dive. All tubes are inserted into the sediment to a depth of six inches. If needed, the core tube may be gently rotated or moved from side-to-side to facilitate insertion. Movement should not suspend the surface sediment. A cap is placed on the top of the core immediately after insertion to prevent disturbance and provide backpressure needed to retain the sediment during retrieval. The diver can then either slide his/her hand under the bottom to pull the tube up and cap the bottom or dig a small hole next to the tube to cap the tube before retrieval.
6. Cores are brought to the surface maintaining the vertical position in which they were collected.
7. Rinse the outside of the tubes with water, then measure and photograph the cores.
8. If the sample is considered acceptable using the criteria listed above, remove the overlying water using a siphon or other similar means, taking care not to remove the flocculent layer. Using a clean stainless steel spoon, collect the top 2 cm of sample. Depending on the length of the tube and the sediment core it may not be possible to reach the sediment surface with a spoon. If the sediment is out of reach, the core tube can be cut with a hack saw one inch above the sediment surface, or the sediment can be pushed up from the bottom until the surface sediment is reachable with a spoon.
9. Repeat the previous procedure for the collection of the next deeper sediment layer (2-4 cm) for archive.
10. Process samples in a manner consistent with the instructions in Section 4.
11. If the sample is not acceptable, remove caps, and rinse clean of sediment away from sampling site and redeploy.

Replicate Sample Locations

Replicate samples should not be collected in the same sampling location. The sampler operator should collect each replicate from opposite sides of the boat moving in a stepwise fashion up the sides of the boat in an up-current direction. Each sampling station should be at least 3 feet apart. In the event that more replicates are required after the operator reaches the most up-current location on the boat, the boat should be moved at least 30 ft in an up-current direction and the process repeated.

4. SAMPLE PROCESSING.

The samplers should be maintained in an upright position until the surface sediments have been removed. Care should be taken to minimize disturbance of surface sediments.

Dewatering

On the boat, the sample should be allowed to settle for at least 1 minute. It is recommended that the dewatering occur before moving to another station as the motion of the boat can disturb the sediment. The overlying water should be siphoned off and returned to the sampling area. A concerted effort should be made to avoid the removal of flocculent material.

Surface Sample

A decontaminated spoon should be used to scoop the sediment layer out of the sampler and into a stainless steel mixing bowl.

Subsurface Sediment

Once the surface sediment is removed from a core sampler, the remaining sediment will generally be returned to the water. If additional samples are to be collected at the same station, care should be taken to release unused sediment after all samples are collected or release them into the water down current of the sample collection area. If subsurface sediments will be retained for analysis, use a hack saw to cut the core within the top inch of the remaining sediment. Alternatively, a core extruder can be used to facilitate sample collection. The core should be capped and maintained in an upright orientation until frozen for shipment and storage.

Resource Area Composites

Oyster bed sediment samples are typically collected in locations more than 100 ft apart to better represent the exposure point concentration for the resource area. The field team will be given the coordinates for up to eight sediment sampling locations for each study grid. The field team will visit the sample locations in the order specified, with the goal of collecting four individual samples per cell that may then be combined into two composite samples by the analyzing laboratory. The field team will collect approximately equal volumes of sediment at each location, which will be transferred into sample containers and sent to the lab under chain-of-custody.

Sample Homogenization

The sample should be thoroughly mixed for minimum of one (1) minute and should be homogenous in appearance. In particular, no separated water should be present and there should not be significant streaking. Water may separate, particularly in sand samples. Samples should be remixed if necessary.

Sample Splits

The sediment sample should be divided into two sample containers with a capacity of 250 mL. These containers should be filled to 75% capacity to assure enough sample container capacity during frozen storage.

Decontamination

Wash the spoon and stainless steel mixing bowl with dilute Alconox between samples. Thoroughly rinse all surfaces with deionized water.

Labels

The sample container lids should be labeled immediately before filling with the sample location, date and time. An identical record should appear in the field log with any additional information that

is required under the QAPP. The samples will be transferred with a complete chain of custody to the sample intake team as soon as possible.

The analytical parameters will be assigned and stored as described in Table A-3.

Table A-3. Sample Analyses and Sizes.

Test	Reference Method	Sample Size (g wet) Min	Ideal	Collected (mL)	Sample Storage
Grain Size	ASTM 422-63	100	175	187.5	Refrigrerated
TPH/THC	EPA 8015	50	75	187.5	Frozen
PAH	EPA 8270				
Biomarkers	EPA 8270				
TOC	EPA 9060	0.5	2		
Total		150.5	252	375	

5. QUALITY CONTROL

The quality control procedures for this method include three primary parts: staff training, equipment blanks, and field duplicates.

Staff Training

The field sampling staff shall read this SOP and be shown how to properly operate all field sampling equipment by an experienced staff member before performing these tasks without supervision. This training will include specific guidelines for how to accurately, precisely, and safely load and trigger the sampler without placing any appendages in the mouth of the sampler.

Equipment Blanks

Whenever possible, the field team should use disposable sampling equipment (e.g. polycarbonate core liners and gloves) during the collection of each sample. It is recommended that one equipment blank be collected for each 20 samples. The equipment blank consists of distilled or deionized water pored over reused sampling equipment (e.g., stainless steel bowls and van Veen grab samplers). The rinsate shall be collected in a 1 L bottle. This water blank should be analyzed by the same hydrocarbon testing methods requested for the associated sediment samples

Field Duplicates

It is recommended that each field team collect one field duplicate for each 20 samples. The field duplicate should be collected in a manner identical to the original sediment sample (e.g., a composite of two sampling locations within an oyster bed). The field duplicate should be analyzed by the same hydrocarbon testing methods requested for the parent sediment samples.

F. SOP for Gonadal Condition

1. Objective.

Determine the reproductive condition of oysters at each sampling site. These data can then be compared with larval supply and settlement data to determine potential impact of oil contamination on recruitment of oysters.

2. Field procedures.

i. Collect at least 10 market-sized oysters (>74mm) from each site for determination of condition index (CI), gonadal index (GI) and sex. See Section D for the SOP regarding tissue collection.

ii. Place oyster in a prelabeled bag and place in a cooler with ice.

3. Lab procedures (within 72 hours)

i. Select 10 market-sized oysters from the sample, and wash, scrap and scrub to remove mud and attached biota.

ii. Measure (to the nearest mm) the length (umbo-to-bill) of each oyster.

iii. Remove and retain the right valve.

iv. Measure (to the nearest 0.1 mm) adductor muscle length.

v. Detach the left valve from the adductor muscle, and combine with the right valve; matched valves are blotted dry and weighed.

vi. Blot and weigh (to the nearest 0.1 g) oyster meat to obtain wet weight.

vii. Bisect the oyster, measure (to nearest 0.1 mm) the width of the gonad and blot gonadal material onto the slide for determination of sex. (As a response to stress, oysters may resorb gonadal material or females may revert to the energetically less demanding life of the male.)

viii. CI is determined as the (blotted) wet weight of the oyster meat divided by (blotted) shell weight.

ix. GI index is measured as the width of the gonad, standardized by dividing gonadal width by adductor muscle length.

x. Sex is determined by bisecting the oyster at the plane of the gills and labial palps, and blotting gonadal material on a glass slide for microscopic examination (Soniat and Ray, 1985). Sex is determined as male (motile sperm), female (eggs), undifferentiated (unknown), and both, or hermaphroditic, and expressed as a population statistic, percent female.

These laboratory techniques are non-destructive to the oyster tissue and are potentially available to collaborative studies which measure the hydrocarbon concentration of oyster meats. The objective of this research is to access differences between impacted and un-impacted sites in recruitment, size-specific mortality, percent female, and oyster condition (CI) and reproductive state (GI).

G. SOP for Oyster Disease Analysis

Dermo Technique

- Use 10 commercial-size oysters (>75mm). See Section D for the SOP regarding tissue collection. The same oysters used for Gonadal Condition analysis will also be used for oyster disease analysis.
- Measure shell height (umbo-to-bill distance) to the nearest mm
- Remove the right valve
- Remove a piece of mantle tissue (~6mm^2) from the right side of the oyster at the anterior margin of the mantle just posterior to the labial palps
- Fortify each tube of fluid thioglycollate (FT) medium (FTM)with 200 units of mycostatin (nystatin) and 200 micrograms of chloromycetin (chloramphenicol) just prior to use (see below for medium preparation)
- Place the tissue in a tube of FTM
- Incubate in the dark at room temperature for a week
- Place the tissue on a glass slide and add 3 drops of diluted Lugol's solution. Flatten the tissue with a blunt probe to get a thin, well-stained preparation. Press a cover slip firmly over the tissue to flatten it more. Remove excess Lugols with absorbent paper
- Examine stained tissue microscopically at 25X and 100X for brown, blue or black spheres (Ray 1966)
- Rate the level of infection as a disease code number according to the criteria of Craig et al. (1989), where 0 is uninfected and 5 is heavily infected
- Calculate percent infection (PI), weighted prevalence (WP) and infection intensity (II) as:

  PI = (number of infected oysters/number of oysters tested) x 100

  WP = sum of disease code numbers/number of oysters tested

  II = sum of disease code numbers/number of infected oysters

Medium preparation

- Rehydrate 29 grams of FTM with 1 liter of distilled water containing 20 grams of NaCl
- Dispense rehydrated medium in 10ml volumes into glass culture tubes and autoclave
- Store sterile tubes of medium in the dark at room temperature until needed.

Appendix B. Oyster Sample ID Naming Convention

NOAA NRDA Sample Format:

- **LocationCode – DateCode - Matrix Leader# Sample#**
    - 6-digit Location code (from maps located on FTP site. These should be the NRDA Grid location code rather than the SCAT location code);
    - 5-digit date: year letter and mmdd (A=2010, B=2011);
    - Matrix letter (T = Tissue or S = Sediment);
    - 2 or 3-digit leader code; and
    - 2-digit sample number.
- EXAMPLE: **LAAM24-A0502-TA102**
    - LocationCode = LAAM24;
    - Date = 5/2/2010;
    - Matrix = Tissue;
    - Leader code = A1;
    - Sample # = 02.

Additional Information for Oysters:

Field Teams

- We will be numbering each sample sequentially **by type**. This information will go in the "Sample #" section at the end of the NOAA NRDA required tag.
- In addition, because there are several different tissue sample types, we will add an identifier after the sample number that will indicate the sample type for tissue samples.
    - DR = dredge sample **(See Next Page)**;
    - L = larval sample (add LS for surface, LM for mid water column or LB for bottom); and
    - SP = settlement plate.
    - Examples: **LAAM24-A0502-T10302DR; LAAM24-A0502-T10303L**

- **Dredge Subsamples**
    - Contaminant subsample
        - Add "-CT" to the end of the sample name, e.g., **LAAM24-A0502-T10302DR-CT**
        - Composite across dredges if necessary. In this case, indicate in the "Sample Notes" which dredges the sample is taken from and how many of each size class were retained from each dredge. GPS coordinates should correspond to the center of the entire cell, rather than a specific dredge.
    - Gonad/Disease subsample
        - Add "-GD" to the end of the sample name, e.g., **LAAM24-A0502-T10302DR-GD**. GPS coordinates should correspond to the center of the entire cell.
- All additional information describing the samples will be recorded in the "Sample Notes" field of the NOAA NRDA sample collection forms. This additional information differs by sample type.
    - Dredge Oysters
        - Cell number
    - Larval Samples
        - Cell number
        - Depth (Surface, Middle, or Bottom)
    - Settlement Plates
        - Cell number
    - Sediment Samples
        - Cell number

Lab Teams

- **Larval samples**
    - Retain same sample name; "Sample Notes" field of the NOAA NRDA sample collection forms should indicate which samples are intended for manual counts versus PCR.

Appendix C. Oyster Transition Plan Field Forms

Team Leader Code: _____

Survey Team ID: _____

NRDA Oyster Site Form – Recruitment Sampling (Version 1)

One form should be used for each assigned site.

1. Site Descriptors

Site Name_____ Cell Number_____

Time:_____ Date:_____

Habitat Setting (check one): ____ Intertidal _____ Subtidal (Depth: _____)

Overall Reef condition:_____

_____

2. Physical/Chemical Parameters

Air Temperature: _____ Surface Temperature: _____ Bottom Temperature_____

Surface Dissolved Oxygen (%):_____ Bottom Dissolved Oxygen (%):_____

Surface D.O. (mg/L):_____ Bottom D.O. (mg/L):_____

Surface Salinity (ppt):_____ Bottom Salinity (ppt):_____

Weather Conditions _____

Oiled Condition (check one): _____ none _____ Sheen _____ Scattered Deposits

_____ Surface substantially covered _____ Surface completely covered_____ Deep Deposits

3. Settlement Plate Retrieval **Example Sample#: LAAM24-A0502-T6004SP**

Crab Pot 1 **Corner of Gridcell: _____**

Sample #'s:_____

Lat_____ Long_____ Time:_____

Crab Pot 2 **Corner of Gridcell: _____**

Sample #'s:_____

Lat_____ Long_____ Time:_____

4. Settlement Plate Deployment

Crab Pot 1 **Corner of Gridcell: _____**

Lat_____ Long_____ Time:_____

Crab Pot 2 **Corner of Gridcell: _____**

Lat_____ Long_____ Time:_____

5. Larvae Samples Example Sample#: LAAM24-A0502-T6008L

Bottom = Lat_____ **Long**_____ **Sample #:** _____ **Depth (m):**_____

Time:_____ **Initials of Sampler**_____

Bottom = Lat_____ **Long**_____ **Sample #:** _____ **Depth (m):**_____

Time:_____ **Initials of Sampler**_____

Middle = Lat_____ **Long**_____ **Sample #:** _____ **Depth (m):**_____

Time:_____ **Initials of Sampler**_____

Top = **Lat**_____ **Long**_____ **Sample #:** _____ **Depth (m):**_____

Time:_____ **Initials of Sampler**_____

Top = **Lat**_____ **Long**_____ **Sample #:** _____ **Depth (m):**_____

Time:_____ **Initials of Sampler**_____

****

Responsible Party Rep: _____ _____ _____ **Date**_____
(Name) (Agency) (Signature)

State Rep: _____ _____ _____ **Date**_____
(Name) (Agency) (Signature)

Federal Rep: _____ _____ _____ **Date**_____
(Name) (Agency) (Signature)

Data Entry: _____ _____ _____ **Date**_____
(Name) (Agency) (Signature)

Version 1
10/17/2010

Team Leader Code: _____ **Survey Team ID:** _____

OYSTER TRANSITION PLAN

NRDA Oyster Site Form – Recruitment Sampling (Version 2)

One form should be used for each assigned site.

1. Site Descriptors

Site Name_____ **Cell Number (GCID)** _____

Time:_____ **Date:**_____

Habitat Setting (check one): ____ **Intertidal** _____ **Subtidal (Depth:** _____**)**

Overall Reef condition: _____

_____

2. Physical/Chemical Parameters

Air Temperature: _____ **Surface Temperature:** _____ **Bottom Temperature**_____

Surface Dissolved Oxygen (%):_____ **Bottom Dissolved Oxygen (%):**_____

Surface D.O. (mg/L):_____ **Bottom D.O. (mg/L):**_____

Surface Salinity (ppt):_____ **Bottom Salinity (ppt):**_____

Weather Conditions _____

Oiled Condition (check one): _____ **none** _____ **Sheen** _____ **Scattered Deposits**

_____ **Surface substantially covered** _____ **Surface completely covered**_____ **Deep Deposits**

3. Settlement Plate Retrieval **Example Sample#: LAAM24-A0502-TA104SP**

Crab Pot 1 **Corner of Gridcell:** _____

Sample #'s:_____

Lat_____ **Long**_____ **Time:**_____

Crab Pot 2 **Corner of Gridcell:** _____

Sample #'s:_____

Lat_____ **Long**_____ **Time:**_____

4. Settlement Plate Deployment

Crab Pot 1 **Corner of Gridcell:** _____

Lat_____ **Long**_____ **Time:**_____

Crab Pot 2 **Corner of Gridcell:** _____

Lat_____ **Long**_____ **Time:**_____

* The YSI instrument should be calibrated every day using the conductivity calibration solution provided by Dade Moeller at intake. Calibrate the dissolved oxygen sensor daily with water-saturated air, and replace with new D.O. membranes as necessary. The User Manual advises that the membrane cap be changed every 60 days during regular use. Record daily calibrations in the field log book.

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5. Larvae Samples Example Sample#: LAAM24-A0502-TA108LB

Bottom = Lat_____ **Long**_____ **Sample #:** _____ **Depth (m):**_____

Time:_____ **Initials of Sampler**_____

Bottom = Lat_____ **Long**_____ **Sample #:** _____ **Depth (m):**_____

Time:_____ **Initials of Sampler**_____

Middle = Lat_____ **Long**_____ **Sample #:** _____ **Depth (m):**_____

Time:_____ **Initials of Sampler**_____

Top = **Lat**_____ **Long**_____ **Sample #:** _____ **Depth (m):**_____

Time:_____ **Initials of Sampler**_____

Top = **Lat**_____ **Long**_____ **Sample #:** _____ **Depth (m):**_____

Time:_____ **Initials of Sampler**_____

****

Responsible Party Rep: _____ (Name) _____ (Agency) _____ (Signature) **Date**_____

State Rep: _____ (Name) _____ (Agency) _____ (Signature) **Date**_____

Federal Rep: _____ (Name) _____ (Agency) _____ (Signature) **Date**_____

Data Entry: _____ (Name) _____ (Agency) _____ (Signature) **Date**_____

Version 2
11/23/2010

Team Leader Code: _____ **Survey Team ID: _____**

OYSTER TRANSITION PLAN
NRDA Oyster Site Form – Dredge Sampling

One form should be used for each assigned site.

1. Site Descriptors

Site Name_____ Cell Number (GCID) _____

Time:_____ Date:_____

Habitat Setting (check one): ____ Intertidal _____ Subtidal (Depth: _____)

Overall Reef condition:_____

_____

2. Physical/Chemical Parameters*

Air Temperature: _____ Surface Temperature (°C): _____ Bottom Temperature (°C) _____

Surface Dissolved Oxygen (%):_____ Bottom Dissolved Oxygen (%):_____

Surface D.O. (mg/L):_____ Bottom D.O. (mg/L):_____

Surface Salinity (ppt):_____ Bottom Salinity (ppt):_____

Weather Conditions _____

Oiled Condition (check one): _____ none _____ Sheen _____ Scattered Deposits

_____ Surface substantially covered _____ Surface completely covered_____ Deep Deposits

3. Dredge Sampling:

Dredge 1

Start of Dredge 1 = Lat_____ Long_____ Time:_____

End of Dredge 1 = Lat_____ Long_____ Time:_____

Length of Dredge Pull (Minutes, seconds): _____

Abundance	< 25 mm SH (Spat)	26 – 74 mm SH (Seed)	> 75 mm (market size)
Live Oysters (Total)			
Dead Oysters (Boxes)			
Retained for CT sample	----------		
Retained for GD sample	----------		
Other Species (List below)			
Oyster drills:			

Notes regarding resource :

_____

_____

_____

* The YSI instrument should be calibrated every day using the conductivity calibration solution provided by Dade Moeller at intake. Calibrate the dissolved oxygen sensor daily with water-saturated air, and replace with new D.O. membranes as necessary. The User Manual advises that the membrane cap be changed every 60 days during regular use. Record daily calibrations in the field log book.

Dredge 2

Start of Dredge 2 = Lat_____ Long_____ Time:_____

End of Dredge 2 = Lat_____ Long_____ Time:_____

Length of Dredge Pull (Minutes, seconds): _____

Abundance	< 25 mm SH (Spat)	26 – 74 mm SH (Seed)	> 75 mm (market size)
Live Oysters (Total)			
Dead Oysters (Boxes)			
Retained for CT sample	----------		
Retained for GD sample	----------		
Other Species (List below)			
Oyster drills:			

Notes regarding resource :

_____

_____

Dredge 3

Start of Dredge 3 = Lat_____ Long_____ Time:_____

End of Dredge 3 = Lat_____ Long_____ Time:_____

Length of Dredge Pull (Minutes, seconds): _____

Abundance	< 25 mm SH (Spat)	26 – 74 mm SH (Seed)	> 75 mm (market size)
Live Oysters (Total)			
Dead Oysters (Boxes)			
Retained for CT sample	----------		
Retained for GD sample	----------		
Other Species (List below)			
Oyster drills:			

Notes regarding resource :

_____

_____

4. Additional Dredges:

NOTE: A single contaminant sample and single gonad/disease sample should be collected for each site. The contaminant sample should consist of 10 market size oysters. The gonad/disease sample should also consist of 10 market size oysters. In addition to the three enumerated dredges, pull additional dredges until the required volume of oysters is collected or two hours has passed. The resource in these dredges does not need to be enumerated by size category. If resource is scarce, follow the procedure in the SOP (Oyster Transition Sampling Plan, Appendix D) for allocating resource to contaminant and gonad/disease samples.

Dredge 4 (If necessary)

Start of Dredge 4 = Lat_____ Long_____ Time:_____

End of Dredge 4= Lat_____ Long_____ Time:_____

Length of Dredge Pull (Minutes, seconds): _____

# of market Oysters retained: _____ # of seed Oysters retained: _____

Dredge 5 (If necessary)

Start of Dredge 5 = Lat_____ Long_____ Time:_____

End of Dredge 5= Lat_____ Long_____ Time:_____

Length of Dredge Pull (Minutes, seconds): _____

# of market Oysters retained: _____ # of seed Oysters retained: _____

Dredge 6 (If necessary)

Start of Dredge 6 = Lat_____ Long_____ Time:_____

End of Dredge 6= Lat_____ Long_____ Time:_____

Length of Dredge Pull (Minutes, seconds): _____

# of market Oysters retained: _____ # of seed Oysters retained: _____

Dredge 7 (If necessary)

Start of Dredge 7 = Lat_____ Long_____ Time:_____

End of Dredge 7= Lat_____ Long_____ Time:_____

Length of Dredge Pull (Minutes, seconds): _____

# of market Oysters retained: _____ # of seed Oysters retained: _____

Dredge 8 (If necessary)

Start of Dredge 8 = Lat_____ Long_____ Time:_____

End of Dredge 8= Lat_____ Long_____ Time:_____

Length of Dredge Pull (Minutes, seconds): _____

# of market Oysters retained: _____ # of seed Oysters retained: _____

5. Sample IDs:

Contaminant Sample *Example: LAAM24-A1120-T10302DR-CT*

Contaminant Sample ID: _____

Number of market Oysters: _____ Number of seed Oysters: _____

Gonad/Disease Sample *Example: LAAM24-A1120-T10302DR-GD*

Gonad/Disease Sample ID: _____

Number of market Oysters: _____ Number of seed Oysters: _____

****

Responsible Party Rep : _____ _____ _____ **Date**_____
(Name) (Agency) (Signature)

State Rep: _____ _____ _____ **Date**_____
(Name) (Agency) (Signature)

Federal Rep: _____ _____ _____ **Date**_____
(Name) (Agency) (Signature)

Data Entry: _____ _____ _____ **Date**_____
(Name) (Agency) (Signature)

Version 1
11/23/2010

Team Leader Code: _____ Survey Team ID: _____

OYSTER TRANSITION PLAN

NRDA Oyster Site Form – Dredge Sampling (Version 2)

One form should be used for each assigned site.

1. Site Descriptors

Site Name_____ Cell Number (GCID) _____
Time:_____ Date:_____
Habitat Setting (check one): ____ Intertidal _____ Subtidal (Depth(m): _____)
Overall Reef condition:_____
_____

2. Physical/Chemical Parameters*

Air Temperature(°C): _____ Surface Temperature (°C): _____ Bottom Temperature (°C) _____
Surface Dissolved Oxygen (%):_____ Bottom Dissolved Oxygen (%):_____
Surface D.O. (mg/L):_____ Bottom D.O. (mg/L):_____
Surface Salinity (ppt):_____ Bottom Salinity (ppt):_____
Weather Conditions _____
Oiled Condition (check one): _____ none _____ Sheen _____ Scattered Deposits
_____ Surface substantially covered _____ Surface completely covered_____ Deep Deposits

3. Dredge Sampling:

NOTE: *Even if no live resource is collected in a dredge, please record the information below.* Remove mud/sediment from dredge to the extent possible, then bag remaining material collected in the dredge and create a sample (e.g., *LAAM24-A1120-T10302DR).* If there is nothing in the dredge other than sediment/mud, please record this in the space below.

Dredge 1 **SAMPLE ID: _____**

Start of Dredge 1 = Lat_____ Long_____ Time:_____

End of Dredge 1 = Lat_____ Long_____ Time:_____

Length of Dredge Pull (Minutes, seconds): _____

Notes regarding resource :

_____

_____

_____

* The YSI instrument should be calibrated every day using the conductivity calibration solution provided by Dade Moeller at intake. Calibrate the dissolved oxygen sensor daily with water-saturated air, and replace with new D.O. membranes as necessary. The User Manual advises that the membrane cap be changed every 60 days during regular use. Record daily calibrations in the field log book.

Dredge 2 **SAMPLE ID: _____**

Start of Dredge 2 = Lat_____ Long_____ Time:_____

End of Dredge 2 = Lat_____ Long_____ Time:_____

Length of Dredge Pull (Minutes, seconds): _____

Notes regarding resource :

_____

_____

Dredge 3 **SAMPLE ID: _____**

Start of Dredge 3 = Lat_____ Long_____ Time:_____

End of Dredge 3 = Lat_____ Long_____ Time:_____

Length of Dredge Pull (Minutes, seconds): _____

Notes regarding resource :

_____

_____

4. Additional Dredges:

NOTE: In addition to the three required dredges, pull additional dredges until the required volume of oysters (over 20 market sized oysters or the equivalent in seed sized oysters) is collected or two hours have passed. If market-sized oysters are not available, the equivalent wet weight tissue from seed-sized oysters can be used for contaminant analysis. The ratio of seed to market-sized oysters is 1.5:1.

Dredge 4 (If necessary) **SAMPLE ID: _____**

Start of Dredge 4 = Lat_____ Long_____ Time:_____

End of Dredge 4= Lat_____ Long_____ Time:_____

Length of Dredge Pull (Minutes, seconds): _____

Notes regarding resource :

_____

_____

Dredge 5 (If necessary) **SAMPLE ID: _____**

Start of Dredge 5 = Lat_____ **Long**_____ **Time:**_____

End of Dredge 5= Lat_____ **Long**_____ **Time:**_____

Length of Dredge Pull (Minutes, seconds): _____

Notes regarding resource :

_____

_____

Dredge 6 (If necessary) **SAMPLE ID:** _____

Start of Dredge 6 = Lat_____ **Long**_____ **Time:**_____

End of Dredge 6= Lat_____ **Long**_____ **Time:**_____

Length of Dredge Pull (Minutes, seconds): _____

Notes regarding resource :

_____

_____

Dredge 7 (If necessary) **SAMPLE ID:** _____

Start of Dredge 7 = Lat_____ **Long**_____ **Time:**_____

End of Dredge 7= Lat_____ **Long**_____ **Time:**_____

Length of Dredge Pull (Minutes, seconds): _____

Notes regarding resource :

_____

_____

Dredge 8 (If necessary) **SAMPLE ID:** _____

Start of Dredge 8 = Lat_____ **Long**_____ **Time:**_____

End of Dredge 8= Lat_____ **Long**_____ **Time:**_____

Length of Dredge Pull (Minutes, seconds): _____

Notes regarding resource :

_____

_____

****

Responsible

Party Rep : _____ _____ _____ **Date**_____

(Name) (Agency) (Signature)

State Rep: _____ _____ _____ **Date**_____

(Name) (Agency) (Signature)

Federal Rep: _____ _____ _____ **Date**_____

(Name) (Agency) (Signature)

Data Entry: _____ _____ _____ **Date**_____

(Name) (Agency) (Signature)

OYSTER TRANSITION PLAN

NRDA Oyster Intake Lab Form – Dredge Enumeration and Sample Generation

One form should be used for each assigned site/grid cell.

1. Site Descriptors

Site Name_____ **Cell Number (GCID)** _____

Habitat Setting (check one): ____ **Intertidal** _____ **Subtidal (Depth:** _____**)**

Time of processing:_____**Date:**_____

2. Dredge Sampling:

Dredge 1 **SAMPLE ID:** _____

Abundance	< 25 mm SH (Spat)	26 – 74 mm SH (Seed)	> 75 mm (market size)
Live Oysters (Total)			
Dead Oysters (Boxes)			
Retained for CT sample	----------		
Retained for GD sample	----------		
Other Species (List below)			
Oyster drills:			

Dredge 2 **SAMPLE ID:** _____

Abundance	< 25 mm SH (Spat)	26 – 74 mm SH (Seed)	> 75 mm (market size)
Live Oysters (Total)			
Dead Oysters (Boxes)			
Retained for CT sample	----------		
Retained for GD sample	----------		
Other Species (List below)			
Oyster drills:			

Dredge 3 **SAMPLE ID:** _____

Abundance	< 25 mm SH (Spat)	26 – 74 mm SH (Seed)	> 75 mm (market size)
Live Oysters (Total)			
Dead Oysters (Boxes)			
Retained for CT sample	----------		
Retained for GD sample	----------		
Other Species (List below)			
Oyster drills:			

3. Additional Dredges:

Dredge 4 (If necessary) **SAMPLE ID:** _____

# of market Oysters collected: _____ **# of seed Oysters collected:** _____

Dredge 5 (If necessary) **SAMPLE ID:** _____

# of market Oysters collected: _____ **# of seed Oysters collected:** _____

Dredge 6 (If necessary) **SAMPLE ID:** _____

# of market Oysters collected: _____ **# of seed Oysters collected:** _____

Dredge 7 (If necessary) **SAMPLE ID:** _____

# of market Oysters collected: _____ **# of seed Oysters collected:** _____

Dredge 8 (If necessary) **SAMPLE ID:** _____

# of market Oysters collected: _____ **# of seed Oysters collected:** _____

4. Sample Generation:

Contaminant Sample

Contaminant Sample ID: _____

Total Number of market Oysters: _____ **Total Number of seed Oysters: _____**

Oysters contributed from individual dredges:

Dredge 1 Sample ID: _____ # Market:_____# Seed: _____

Dredge 2 Sample ID: _____ # Market:_____# Seed: _____

Dredge 3 Sample ID: _____ # Market:_____# Seed: _____

Dredge 4 Sample ID: _____ # Market:_____# Seed: _____

Dredge 5 Sample ID: _____ # Market:_____# Seed: _____

Dredge 6 Sample ID: _____ # Market:_____# Seed: _____

Dredge 7 Sample ID: _____ # Market:_____# Seed: _____

Dredge 8 Sample ID: _____ # Market:_____# Seed: _____

Gonad/Disease Sample

Gonad/Disease Sample ID: _____

Total Number of market Oysters: _____ **Total Number of seed Oysters: _____**

Oysters contributed from individual dredges:

Dredge 1 Sample ID: _____ # Market:_____# Seed: _____

Dredge 2 Sample ID: _____ # Market:_____# Seed: _____

Dredge 3 Sample ID: _____ # Market:_____# Seed: _____

Dredge 4 Sample ID: _____ # Market:_____# Seed: _____

Dredge 5 Sample ID: _____ # Market:_____# Seed: _____

Dredge 6 Sample ID: _____ # Market:_____# Seed: _____

Dredge 7 Sample ID: _____ # Market:_____# Seed: _____

Dredge 8 Sample ID: _____ # Market:_____# Seed: _____

****

Lab Team Leader:	_____	_____	_____	_____
	(Name)	(Agency)	(Signature)	(Date)
Analyzed By:	_____	_____	_____	_____
	(Name)	(Agency)	(Signature)	(Date)
Data Entry:	_____	_____	_____	_____
	(Name)	(Agency)	(Signature)	(Date)